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(54) **New Ig Extracts having An Immunomodulator Activity**

(57) The present invention concerns a process for the preparation of Ig extracts from human polyvalent intravenous immunoglobulins (IgIV) which would appear to be more particularly responsible for the immunomodulator effect observed when treating certain autoimmune diseases. The invention deals with Ig extracts that have a reactivity towards IgM's, IgG F(ab')2's, or DNP haptene and no or only little reactivity towards nonself antigens, i.e. Ig extracts that have idiotypical type

interactions between themselves (connected extract) or that comprise natural antibodies that react with DNP haptene. These extracts show a polyreactivity towards given autoantigens.

Description

[0001] The present invention concerns a process for the preparation of Ig extracts from human polyvalent intravenous immunoglobulins (IgIV) which would appear to be more particularly responsible for the immunomodulator effect observed when treating certain autoimmune diseases. The invention deals with Ig extracts that have a reactivity towards IgM's, IgG F(ab')2's, or DNP haptene and no or only little reactivity towards nonself antigens, i.e. Ig extracts that have idiotypical type interactions between themselves (connected extract) or that comprise natural antibodies that react with DNP haptene. These extracts are polyreactive towards given autoantigens.

[0002] Preparations of IgIV have been used for a number of years for the treatment of multiple pathological states. The major indications may be grouped into three therapeutic targets

- Primary or secondary immunitary deficits
- Treatment of certain autoimmune diseases
- Infectious complications and disease of graft against the host after grafting hematopoietic allogenic cells

[0003] In the case of immunitary deficits, IgIV's constitute a substitute treatment that makes it possible to bring IgG's whose plasmatic concentration in patients is not sufficient to neutralize the development of viral or bacterial infections.

[0004] In autoimmune diseases, the effectiveness of IgIV's is associated with complex immunomodulator effects. As prescribed within the framework of bone marrow grafts, IgIV's correspond to a substitute treatment until immunological reconstitution of the grafted subjects is achieved and have an immunomodulator effect on the graft disease against the host.

[0005] IgIV's are prepared from a plasma pool originating from many thousands of donors. They show a distribution in sub-classes and antibody specificities that correspond to that of the population in general. Thus, IgIV's may be considered as a product that contains the totality of the natural antibody repertory and antibodies directed against exterior antigens and autoantigens.

[0006] The IgIV immune regulation concept was largely developed since the demonstration of their efficiency in thrombopenic autoimmune purpura (PTA) in 1981 (1). IgIV's were thereafter used in numerous autoimmune or inflammatory pathologies. Certain indications for which the efficiency of IgIV's was clearly established, are officially recognized by regulatory boards. These include PTAI, the Kawasaki disease in which they very efficiently prevent aneurysmal complications (2,3), the graft of hematopoietic allogenic cells where they modulate the reaction of the graft against the host (4) and more recently, Birdshot's retino-choroiditis, where they improve visual acuity and sometimes allow to reduce corticotherapy (5). Other indications are considered by the experts as being justified. For example, some

cytopeniae where IgIV's bring about a rapid but often transitory improvement (6), and homophiliae with inhibitors (autoantibody anti-factor VIII) where in return the improvement may be permanent (7,8). Contradictory results have been obtained in repetitive abortions with encouraging success rates in certain series (9,10).

[0007] For the last ten years or so, IgIV's have shown a very important progress in neurology as a result of multicentric controlled studies with quantitative (neurological scores) and qualitative (improved number of patients) criteria of efficiency. Thus, in the Guillain-Barré disease of the adult, IgIV's are as effective as plasmatic exchanges and are better tolerated (11, 12). They are recommended as a first instance in the children forms (13). They are more effective versus placebo in demyelinating chronic inflammatory polyneuropathies (14) and in dermatomyositis (15). They are also effective and better tolerated than plasmatic exchanges during an acute crisis of myasthenia (16). Finally, a study versus placebo has demonstrated the efficiency of IgIV's in the alternating forms of remissions and outbreaks of multiple sclerosis (17).

[0008] Many mechanisms have been proposed to explain the diversity of action of the IgIV's (18)

- Blocking of the Fc receptors at the surface of macrophages, monocytes, neutrophiles and eosinophiles.
- Neutralization of circulating antibodies with anti-idiotype antibodies.
- Inhibition of the harmful effects due to the activation of the complement.
- and/or the selection of the immunitary repertories by interaction with lymphocytes T and B.

[0009] These mechanisms could explain the premature and extended effects of IgIV's.

[0010] An Ig extract (so-called connected) may be purified on an affinity column in which the IgIV F(ab')2's or whole IgG's have been coupled to Sepharose balls (19 and 20). The IgM's contained in the serum of normal individuals are linked to the F(ab')2 fragments of the autologue IgG's and inhibit the association of these IgG's with autoantigens (21). IgM's contribute to regulate the natural autoreactivity of IgG's through idiotypic type interactions (21). These Ig's or their F(ab')2's can inhibit the linkage of some antibodies to their antigens as this has been shown by means of tests carried out in vitro (22). The connected extract of Ig obtained from IgIV's would appear to contain in particular antibodies that recognize anti-idiotypic determinants that are present in the IgG or IgM antibodies that can neutralize themselves and modify the function and the dynamic of the idiotypic network (23). On the other hand, an extract of Ig characterized in that it reacts with DNP haptene is described as containing polyreactive and autoreactive natural antibodies (24).

[0011] Other documents describe the general principle involved to obtain connected extracts. Among these documents, Patent Application WO 98/26086 which relates to a process for preparing a composition that is purified from antibodies comprising anti-idiotypic antibodies may be cited, said process consisting in the adsorption of a pool of IgG's on a solid substrate containing an idiotypic determinant of an

autoantibody, and an elution. EP 293 606 describes a general method for the purification of an antibody X by idiotypic/anti-idiotypic interaction comprising the following steps:

a) binding an antibody Y on a solid support, said antibody recognizing idiotype X,
b) contacting a sample containing an antibody X with the solid support in a suitable buffer,
c) elution, and d) recovery of the purified antibody X.

WO 97/19113 relates to the use of antio-idiotype monoclonal antibodies of the IgG type as immunoregulator of the immunitary response, in particular for the treatment of autoimmune diseases.

[0012] Actually, the tolerance and effectiveness of the commercial polyvalent IgG's, such as TEGELINE® (LFB, France) are well known in particular in the treatment of PTI, the Kawasaki disease and retino-choroiditis of the "Birdshot" type, which are pathologies for which AMM's have been obtained. However, present posologies in these indications are important, and the delivery system remains awkward and complicated (perfusions for many hours in the hospital). The problem therefore consists in preparing an active extract in autoimmune pathologies, so as to make the preparation more efficient and easier to use.

[0013] The objective at the basis of the present invention is therefore to obtain specific Ig's allowing dose decreases, with a maintained and even increased effectiveness, a better tolerance, and whose delivery system is more simple. It has been shown that it is possible to prepare extracts complying with the problems previously mentioned by preparing them from Ig pools so that they have an anti IgM, anti IgG F(ab')2 or anti DNP reactivity, little or no reactivity towards nonself antigens, and/or that show some polyreactivity towards certain autoantigens.

Description

[0014] Thus, the present invention concerns the purification of the Ig's contained in polyvalent IgIV's that would be particularly responsible for the immunomodulator effect observed during treatment of certain autoimmune diseases. The invention is based on the characteristics of these IgG extracts that possess a reactivity towards IgM's, IgG F(ab')2's or DNP haptene, and no or little reactivity towards tetanic toxoid and the HBs antigen (nonself antigen), i.e. extracts containing Ig's having idiotypic type interactions with one another (connected extract) or comprising natural antibodies. These extracts are polyreactive towards some antigens.

[0015] The preparation of Ig extracts is carried out by affinity chromatography by utilizing the property of these Ig's to recognize one another, to recognize IgM's or to bind to DNP haptene. The raw material used to obtain these extracts is obtained from polyvalent Ig's, for example those that are prepared and marketed by LFB (France), or from any other intermediate extract obtained in the process for preparing polyvalent IgIV's for therapeutic use. The general process for preparing polyvalent IgIV's essentially includes the following steps:

- Extraction of the plasma derived from a pool of donors by precipitation, adsorption and/or filtration and ultrafiltration (obtaining a first PSO 1" extract),
- Treatment with pepsin under acid pH, formulation, distribution and freeze-drying (obtaining TEGELINE® product).
- Another treatment could use chromatography on an anion exchange column, ultrafiltration, obtaining an intermediate extract (called "PSO 2") and heating, ultrafiltration, formulation and distribution (obtaining a liquid IgIV extract).
- Within the scope of the present invention, "polyvalent Ig's" means whole polyvalent IgG's or IgM's, extracts of polyvalent IgG's such as F(ab')2 or F(ab) and any intermediate extract obtained in the process for the manufacture of polyvalent IgIV's.
- **[0016]** A first aspect of the invention concerns an Ig extract that reacts with at least one component selected from IgM's. IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 20 with respect to the activity of the initial polyvalent Ig's, and in that the extract does not react with tetanic toxoid and the antigen HBs with a ratio of concentration lower than 5 with respect to the activity of the initial polyvalent Ig's.
- **[0017]** This Ig extract may consist of an IgG extract or an IgM extract. Preferably it reacts with a component selected from IgM's, IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 40 with respect to the activity of the initial polyvalent Ig's.
- **[0018]** The extract according to the invention may also react with at least one of the autoantigens selected from myosin, actin, tubulin and the basic protein of myelin (MBP) with a ratio of concentration higher than 10, preferably 20 with respect to the activity of the initial polyvalent Ig's.
- **[0019]** Advantageously, the extract reacts with the whole of the autoantigens referred to previously.
- **[0020]** A preferred extract according to the invention may be defined in that it reacts with a component selected from IgM's, IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 40 with respect to the activity of the initial polyvalent Ig's, and in that it also reacts with myosin, actin, and MBP with an average ratio of concentration higher than 20 with respect to the activity of the initial polyvalent Ig's.
- **[0021]** The above mentioned extracts can react with IgM's or IgG F(ab')2's. They may also react with DNP heptene and in this case, they do not react with IgM's and IgG F(ab')2's.
- **[0022]** A second aspect of the invention resides in a process for preparing Ig extracts characterized in that it comprises the following steps:

a) Preparation of an insoluble support on which a component selected from IgG's, polyvalent IgM's and DNP-Lysine are grafted,
b) adsorption of polyvalent Ig's on the support obtained in step a),

c) elution of the Ig's retained on the portion of the immunoglobulins bound to the support so as to collect the extract connected by IgG-IgG or IgM-IgG idiotypic interactions, or elution of the extract interacting with DNP,

d) selection of the extracts having a reactivity towards IgM's, IgG F(ab')2' or DNP haptene, no or little reactivity towards nonself antigens and/or a polyreactivity towards given antigens,

e) selection of the extracts having an inhibiting activity of lymphocite proliferation in mixed culture, preferably with an efficiency 10 to 50 times higher and TEGELINE.

[0023] In this process, the absorbed Ig's may be IgG's or IgM's.

[0024] The obtained Ig extracts are prepared from polyvalent Ig's or any other intermediate extract obtained during the process for manufacturing IgIG's for therapeutic use. These polyvalent Ig's may be IgG's or IgM's.

In polyvalent Ig's, there are natural antibodies that interreact with DNP haptene and antibodies that interreact with the idiotypes expressed by autoantibodies of the type IgG or IgM (connected extract) and that have some autoreactivity. The term "connected extract" as used within the present invention means an extract that has a high percentage of Ig's interacting with one another or with IgG's or IgM's through idiotype-antiidiotype linking.

[0025] The strategy utilized to determine, among the different extracts, the extract(s) having the required properties, i.e. the extracts containing the highest autoreactivity titer and reacting with the largest number of autoantigens, consists in treating them by screening including a plurality of consecutive steps.

[0026] The different in vitro and/or in vivo tests in use allow to select at each step the most active extract on the basis of criteria that are more and more specific.

[0027] The process according to the invention may therefore include steps making it possible to select Ig extracts having given characteristics.

[0028] In this connection, step d) may comprise a determination of the ratio of concentration of reactive antibodies against IgM's, IgG F(ab')2's or DNP haptene that are used for purification.

Step d) may also include a reactivity determination for tetanic toxoid and HBs antigen by taking the ratio of concentration as control value.

Preferably, step d) comprises an ELISA test that is carried out on a panel of antigens selected for example from actin, myosin, MBP and tubulin.

[0029] Step d) of the process according to the invention may additionally comprise a competition test for controling the neutralizing activity of the extracts towards autoantibodies derived from the serum of patients having caught autoimmune diseases, and/or an inhibition test of the mixed lymphocytic reaction with human cells to determine the inhibitor capacity.

This mixed lymphocytic reaction test may comprise the following steps:

- obtaining blood samples from an A donor and a B donor which is not compatible with respect to the antigens of the major histocompatibility (CMH) complex,
- purification of mononucleated cells on ficoll,
- culturing 2.10^5 cells of donor B in the presence of 21.10^5 cells of donor A,
- measuring prolifiration of the cells at day 4 by measuring the incorporation of tritiated thymidin.

Step a) consists of grafting polyvalent IgG or IgM or DNP-Lysine on an insoluble support, for example on a Sepharose®, Trisacryl®, Affiprep®, or Affigel® gel, gels activated with CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde) groups. The Ig's deposited on the solid support obtained in step a) are adsorbed either in the form of polyvalent lyophilized Ig's and solubilized again, or in liquid form, or in the form of intermediate extracts obtained in the course of a process of preparing polyvalent Ig's. The Ig's that are deposited comprise IgG's or IgM's. Advantageously, absortion is carried out under conditions of temperature that vary from 4 ° to 40 °C and in phosphate buffer 20 mM or equivalent comprising NaCl whose concentration can vary from 0 M to 3 M.

Elution of the Ig's retained in step b) are preferably eluted during step c) with an ion buffer that can dissociate the linkage Ag-Ac or DNP-A, for example selected from chaotropes such as glycine—HCl or sodium iodide (NaI) under conditions allowing the pH to vary, preferably between 2.8 and 4.0.

[0030] In a particular embodiment, this process comprises the following steps:

a) Grafting polyvalent IgG or IgM or DNP-Lysine on a solid support or an affinity support (immunoadsorbing) normally used in affinity chromatography. Such supports are well known to those skilled in the art. A Sepharose®, Trisacryl®, Affiprep®, or Affigel® gel, gels activated with CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde) groups may be mentioned.

b) Adsorption of Ig in phosphate buffer 20 mM or equivalent comprising NaCl whose concentration may vary from 0 M to 3 M, on the solid support obtained in step a), deposited either in the form of lyophilized polyvalent Ig's and put back in solution, or in liquid form, or in the form of intermediate extracts obtained in the course of the process of preparing polyvalent Ig's. The Ig's that are deposited comprise IgG's or IgM's .

c) Elution of the Ig's retained in step b) with an ion buffer that can dissociate the Ag-Ac linkage, selected for example from chaotropes such as glycine-HCl or sodium iodide (NaI)) under conditions allowing the pH to vary, preferably between 2.8 and 4.0, and/or by any other equivalent method allowing a rupture of the IgG-IgG, IgG-IgM or Ig-DNP-Lysine linkages so as to obtain Ig extracts having a reactivity profile that is different from that of the polyvalent Ig starter.

d) ELISA measurement of the concentration ratio of reactive antibodies against IgM's, IgG F(ab')2's or DNP or TNP haptene used for purification, tetanic toxoid and HBs antigen reactivity determination by taking the ratio of concentration as control value, and determining

the ratio of concentration in terms of reactivity towards a panel of antigens selected for example from actin, myosin, MBP and tubulin.

As noted previously, an additional step comprising a lymphocytic reaction test may also be included in this process.

[0031] In each case, the extract that is not retained on the different columns may also be used as control in addition to the initial preparation of Ig.

Of course, some parameters of the process may be modified to the choice of the man of the art by means of simple routine experiments. The invention therefore relates to a process as noted above in which the parameters are determined as a function of the extracts that have been previously selected in step d). The point is to define the optimum parameters on the scale of an industrial process according to the invention. Such parameters may be those that characterize the extracts described above. Thus, the process may be adapted to obtaining extracts as described above. In the same manner, the invention aims at an industrial process for the manufacture of extracts that are reactive towards a component selected from IgM's, IgG F(ab')2's and DNP heptene, have none or little reactivity towards nonself antigens and a polyreactivity towards given autoantigens, characterized in that one makes use of steps a), b) and c) described above by complying with or adapting the parameters used during the preparation of the extracts of interest previously selected.

The invention also has as an object the extracts that can be obtained from the process as noted previously.

[0032] The immunomodulating properties of some extracts selected by means of in vitro tests may also be determined in vivo in many animal models of autoimmune diseases and of the graft against host (GVH) after allografts disease.

[0033] Many types of models have been selected as a function of the action mechanism involved.

- Models in which the effecting function is ensured my means of T cells or antibodies.
- Models in which the mechanisms depend on interaction with F(ab')2's or Fc's.

[0034] Two experimental autoimmune diseases in the rat, where the effecting function is ensured by means of T cells, are more particularly selected because they have been described as being sensitive to the administration of IgIV and have the advantage of being able to bring a rapid response with respect to the efficiency of the extracts (the protector effects can be evaluated in about 4 weeks). We are concerned with the following models:

1) Experimental autoimmune uveitis or UAE induced by injecting bovine renal antigen or its immunodominant peptide to Lewis rats
2) Rhumatoid polyarthritis (PR) induced in Dark Agouti rats by injecting bovine collagen of type II.

[0035] In each case, the acuteness of the disease is clinically and/or histopathologically evaluated and many biological parameters such as weight loss, production of antibodies against the autoantigen injected, are measured as time go on.

[0036] An acute GVH model in the rat was added since this disease was described as sensitive to the administration of IgIV. GVH is induced in the hybrid rat (LewisX Brown-Norway) by injecting lymphoid cells originating from Lewis rats. The disease was evaluated by weight loss, presence of erythema and death rate.

[0037] The animal model of autoimmune hemolytic anemia (AHA) which mainly involves the action of antibodies, is close to hemolytic pathologies observed in man. It is induced by injecting red blood cells (GR) of rat to previously splenectomized C3H mice. This test is useful because of the efficacy of IgIV noted in hemolytic anemia in man. The development of anemia is followed by a decrease of the number of GR and the appearance in the serum of the animals of autoantibodies directed against their own GR's.

[0038] The protector effect of TEGELINE®, polyvalent IgG or IgM or any other intermediate product obtained in the course of the process of preparing polyvalent Ig's, is previously tested in these models and the optimal conditions of administration (dose, number, delays and way of injection) are determined. The selected extracts are injected at doses that are five to twenty times weaker than those of TEGELINE® and the effectiveness of these treatments is determined in the different models of autoimmune diseases.

[0039] Moreover, experimental models may be produced by using human cells.

- The humanized SCID/NOD mouse appears as the best model to evaluate the effectiveness in vivo on pathological human cells, of extracts that are preselected with tests on the animal models.

[0040] The models of primary biliary cirrhosis, of myastenia and Hashimoto thyroiditis were selected because the cells from these pathologies have already been grafted with success on SCID mice. Other pathologies could thereafter be selected.

[0041] In a later phase and for the purpose of increasing knowledge on the mode of action of a given extract that is shown to be effective, other complementary models can be used in order to expand the indications of use of extracts derived from IgIV's, such as TEGELINE® or the like.

[0042] Step d) may also additionally comprise one or more in vitro test(s), for example the above described tests.

[0043] Thus, the process according to the invention allows for example to prepare and select extracts whose characteristics are described above.

[0044] Once the extracts of interest have been identified, the parameters of steps a), b) and c) may be used within the scope of an industrial process of manufacturing said extracts. Such a process with adequate parameters according to the previously selected extracts of interest is an additional object of the invention.

[{0045] A complementary aspect of the invention resides in extracts that are capable of being obtained from the process define above.

One should note that the description of the present invention is not limiting, and that equivalent processes and equivalent extracts also constitute the invention.

[0046] The extracts according to the invention have many advantages of which the main ones are the following:

- Decrease of dosages. Since the new proposed product corresponds to an extract that is present in polyvalent Ig's, the quantity of injected Ig having immunomodulating properties is lower than those of the normally prescribed IgIV's. Efficient doses may be reduced by a factor of 5 to 20 and even more. This advantage is considerable since the presently used doses for available polyvalent Ig's are very high: of the order of 1 to 2 g/kg.
- A maintained and even increased efficacy since the product is enriched in immunomodulator Ig's.
- A better tolerance. With lower concentrations, tolerance of the new product is improved. Indeed, actually, it is necessary to take some care when administering IgIV's with, in particular, a slow perfusion of the product for many hours to prevent certain secondary effects, such as for example allergic reactions.
- A simplified prescription. The administration of low doses allows to provide ambulatory treatments which replace present perfusions carried out in hospital environment.

[0047] An additional aspect resides in the use of Ig extracts according to the invention for the preparation of a medicament. This medicament is more particularly adapted for the treatment of automune diseases, GVH, and/or graft rejection after transplantation.

[0048] The extracts according to the invention are useful for the preparation of a medicament intended for the treatment of Kawasaki disease, Birdshot retinochoroiditis, possibly in association with a corticotherapy, for the treatment of certain cytopenia, hemophilia with inhibitors (anti-factor VIII autoantibodies), and/or to prevent or overcome the immunitary cell rejection and/or organ grafts and the development of GVH after allogenic cell graft.

[0050] Reference will be had to the legends of the figures presented hereinafter for the continuation of the description.

Legends

[0051] **Figure 1A-1D:** Evaluation of the properties of an extract obtained from TEGELINE® (solid support based on Affigel grafted with TEGELINE®).

The parameters of the process for preparing this extract are explained more in detail in example 1 hereinafter.

FNA means non absorbed extract.

Figures 1A and 1C illustrate specific reactivity towards IgG F(ab')2's and figures 1B and 1D represent reactivity towards autoantigens.

[0052] Figure 2A-2D: Evaluation of the properties of an extract obtained from TEGELINE® (solid support based on NHS-Sepharose).

The parameters of the process for preparing this extract are explained more in detail in example 2 hereinafter.

Figures 2A and 2C illustrate specific reactivity towards IgG F(ab')2's and figures 2B and 2D represent reactivity towards autoantigens.

[0053] Figure 3A-3D: Evaluation of the properties of an extract obtained from TEGELINE® (solid support based on NHS-AffiPrep with DNP-Lysine).

The parameters of the process for preparing this extract are explained more in detail in example 3 hereinafter.

Figures 3A and 3C illustrate specific reactivity towards IgG F(ab')2's and figures 3B and 3D represent reactivity towards autoantigens.

[0054] Figure 4A-4D: Evaluation of the properties of an extract obtained from TEGELINE® (solid support based on NHS-Sepharose grafted with IgM's).

The parameters of the process for preparing this extract are explained more in detail in example 4 hereinafter.

Figures 4A and 4C illustrate specific reactivity towards IgM's and figures 4B and 4D represent reactivity towards autoantigens.

Figure 5: Evaluation of the capacity of TEGELINE® or of extracts to inhibit linkage between DNA and anti-DNA antibodies derived from a serum of a patient suffering from an erythematous Lupus.

The experimental conditions of the competition test are explained in example 6 hereinafter:

■ 47-EN (anti-DNP);) 47-4EN (anti-DNP); □ Tegeline;) 46-8 EA (anti-Tegeline); ■ 46-9 EA (anti-Tegeline).

[0055] Figure 6: Evaluation of the protector effect of the anti-DNP extract as compared to Tegeline on the development of rhumatoid polyarthritis induced by collagen II in the rat.

This figure represents the evolution of the arthritic score with DNP-LYSINE extracts.

Ways of inducing the disease as well as administration of the products are described in example 7A hereinafter.

[0056] Figure 7: Evaluation of the protector effect of the anti-DNP extract as compared to Tegeline on the development of diabetics induced with cyclophosphgamide in the NOD oral mouse.

The ways of inducing the disease as well as the administration of the products are described in example 7B hereinafter.

[0057] The processes of preparation and evaluation of the activity of IgG enriched extracts having the property of associating with other IgG's in interactions of the idiotypic type are presented more in detail in the following examples.

Example 1: Process according to the invention with TEGELINE® and a solid Affigel support.

[0058] The polyvalent IgG's were coupled with a NHS-Affigel gel at the rate of 21 mg of product per ml of gel. A dose of 20 g of polyvalent IgG's at a concentration of 20 mg/ml was contacted by recirculation in a column with 21 of immunoabsorbent during 4h at 22 °C in PBS. Elution was thereafter carried out in glycine HCl 0.1M pH 3.25 and the eluate was concentrated on an ultrafiltration membrane having a threshold cut of 30kD.

Concentration was measured by nephelemetry. Recovery rate was 0.42% in the eluate and 89% in FNA.

The reactivity concentration ratio towards F(ab')2's of this eluate with respect to the polyvalent IgG starters, was 65.

[0059] This extract has an enriched reactivity with respect to that of polyvalent IgG's towards many autoantigens and an absence of reactivity towards tetanic toxoid and the antigen HBs (see figure 1 and table 1)

Table 1

Tested antigens	Ratio of	concentration	%	recovery
	Eluate	FNA	Eluate	FNA
F(ab')2	65	0.3	28	26
TNP	90	0.7	39	62
Toxoid	1.8	1.1	0.8	106
HBs	2.5	1.4	1.1	132
Actin	63.5	0.7	27	69
Myosin	76	0.6	33	57
MBP	29	1	12	90
Tubulin	80	0.8	34	74

Example 2: Process according to the invention with TEGELINE® and a solid support of NHS-Sepharose

[0060] Polyvalent IgG's were coupled to a gel of NHS-Sepharose at the rate of 10 mg of protein per ml of gel. A dose of 50 mg of polyvalent IgG's at a concentration of 1 mg/ml was contacted by recirculation in a column with 20 ml of immunoabsorbent during 4 h at 22 °C in PBS. The non absorbed portion or FNA was collected and kept at –80 °C. Elution was thereafter carried out in glycine buffer HCl 0.1 M pH 3.5 and the eluate was concentrated by centrifugation on an ultrafiltration membrane having a threshold cut of 30 kD.

The IgG concentration was measured by nephelemetry. The recovery rate is 0.77% in the eluate and 94.7% in FNA.

Reactivity concentration ratio towards the F(ab')2's of this eluate with respect to the starter polyvalent IgG's is 76.

This extract has an enriched reactivity with respect to that of polyvalent IgG's towards many autoantigens and an absence of reactivity towards tetanic toxoid and the antigen HBs (figure 2 and table 2).

Table 2

Table 2

Tested antigens	Ratio of	Concentration	%	Recovery
	Eluate	FNA	Eluate	FNA
TNP	32	0.45	22	39
F(ab')2	76	0.2	51	22
Toxoid	1.3	1	0.9	86
HBs	4.87	1.1	3.3	96.4
Actin	29.5	0.6	20	47
Myosin	35	0.6	24	55
MBP	29	0.7	20	58
Tubulin	22.4	0.6	15	54

Example 3: Process according to the invention with DNP-Lysine and a solid support of NHS-AffiPrep.

[0061] DNP-Lysine was coupled to a gel of NHS-Affiprep at the rate of 4 mg of product per ml of gel. A dose of 60 g of polyvalent IgG's at a concentration of 50 mg/ml was contacted by recirculation in a column with 2 l of immunoabsorbent during 4h at 22 °C in PBS. Elution was then carried out in sodium iodide (KI) 2M at pH 7. After concentration on an untrafiltration membrane having a threshold cut of 30kD, the eluate was desalted against PBS on a Sephadex G 25 column.

Concentration was determined by nephelemetry. The recovery rate is 0.12% in the eluate and 85% in FNA.

Reactivity concentration ratio towards TNP-Ova of this eluate with respect to the starter polyvalent IgG's, is 239.

This extract has an enriched reactivity with respect to that of polyvalent IgG's towards many autoantigens and an absence of reactivity towards tetanic toxoid and the HBs antigen (figure 3 and Table 3)

Table 3

Tested antigens	Ratio of	Concentration	%	Recovery

	Eluate	FNA	Eluate	FNA
TNP	239	0.9	23	94
F(ab')2	2.9	0.9	0.6	92
Toxoid	2.4	1	0.5	104
HBs	3.2	1	0.7	104
Actin	117	1.1	24	120
Myosin	83	1.2	17	129
MBP	63	1	13	102
Tubulin	137	1.5	28	152

Example 4: Process according to the invention with polyclonal IgM's and a solid support of NHS-Sepharose.

[0062] Human polyclonal IgM's (90% purity) were coupled to a gel of NHS-Sepharose at the rate of 10 mg of proteins per ml of gel. A dose of 50 mg of polyvalent IgG's at a concentration of 1 mg/ml was contacted with 20 ml of immunoadsorbent during 4 h at 22 °C in PBS. The non adsorbed extract or FNA was collected and kept at –80 °C. Elution was then carried out in glycine HCl O.1 M pH 3.5 buffer and the eluate was concentrated by centrifugation on an ultrafiltration membrane having a threshold cut of 30 kDa. IgG concentration was measured by nephelemetry. Recovery rate is 0.20% in the eluate and 98.7 in FNA. Reactivity ratio of concentration towards IgM's of this eluate with respect to the starter polyvalent IgG's, is 64.

This extract has an enriched reactivity with respect to that of polyvalent IgG's towards many autoantigens and an absence of reactivity towards tetanic toxoid and the HBs antigen (figure 4 and Table 4)

Table 4

Tested antigens	Ratio of	Concentration	%	Recovery
	Eluate	FNA	Eluate	FNA
TNP	71.5	0.5	13	44.5
IgM	64	1.2	11.4	106
F(ab')2	24.5	0.7	4.5	67
Toxoid	1.8	0.8	0.3	76
HBs	< threshold	0.8	< threshold	76
Actin	52	0.3	9	33
Myosin	54	0.6	10	54
MBP	39.5	0.5	7	50

Tubulin	58	0.7	10	62

Example 5: Inhibition of proliferation of human lymphocytes in MLC

[0063] Lymphocytes from a donor A and a donor B which are incompatible with respect to HLA molecules were separated on ficoll and cultured at a concentration of 2X 10^5 per well in PPM1 1640 to which 10% of fœtal calf serum was added. Decreasing concentrations of Tegeline, of Fc or F(ab')2 extracts of Tegeline or different extracts presented in examples 1 to 4 were added to the medium. After 4 days culture at 37 °C in CO_2 atmosphere, 1 µCi = 37 KBq of tritiated thymidine is added during the last 6 h of culture. Measuring the rate of incorporation of tritiated thymidine human cells which represents proliferation, is carried out by means of a scintillation β counter. The percentage of inhibition of proliferation of the lymphocytes in the presence of different components added to the culture is calculated with respect to the proliferation of the mixed cells of donors A and B. Table 5 presents the results in terms of dose in µg/ml of extracts or products capable of giving a 50% inhibition of cell proliferation. The extracts presented in examples 1 to 4 can inhibit proliferation of lymphocytes in mixed culture with an efficacy 10 to 50 times greater that that of Tegeline.

Table 5

Inhibition through TEGELINE® and extracts from proliferation of human lymphocytes in mixed culture

Reference extracts	Affinity support	Dose in µm/ml giving 50% proliferation inhibition	
		Experiment 1	**Experiment 2**
Tegeline®	NA	160	80
Fc of Tegeline®	NA	1000	NT
Tegeline®F(ab')2	NA	NT	250
Example 1	AffiGel NHS Tegeline	7	NT
Example 2	Sepharose NHS Tegeline	5	NT
Example 3	AffiPrepNHS DMP-Lysine0	9	2
Example 4	Sepharose NHS IgM	2.5	-

NA = not applicable

NT = not tested

Example 6: Competition test of extracts towards pathogenous antigens.

[0064] Tangeline or the anti-Tegeline extracts prepared according to example 2 or the anti-DNP extracts prepared according to example 3 are incubated, in the presence of biotinated anti-DNA antibodies originating from a patient having erythematous Lupus, on a microfiltration plate covered with DNA. The percentage of inhibition of the linkage of the biotinated anti-DNA antibody to DNA is measured as a function of the concentration of Tegeline or the added extracts. The results presented in figure 5 show that the anti-DNP extracts inhibit about ten times more proliferation than Tegeline for the same concentration. Anti-Tegeline extracts on the contrary promote linkage of pathogenous antibodies to DNA by providing idiotypical type interactions.

Example 7: Clinical Applications

[0065] The autoreactivity enriched extracts which appear effective in experimental models of autoimmune diseases are intended to be used in the treatment of a number of pathologies where IgIV's have been shown to possess a therapeutic action, and in particular against automune diseases, GVH and graft reject after transplantation.

[0066] **Example 7A:** Effects of anti-DNP extracts as compared to Tegeline on the development of rhumatoid polyarthritis induced by collagen II in the rat.

[0067] Extracts enriched in autoreactivity that are derived from the elution of polyvalent IgG's of a NHS-Affiprep gel coupled to DNP-Lysine (fig 3 and example 3) were injected *ip* at different doses to rats having received collagen II to induce the development of a rhumatoid polyarthritis. Protection efficacy against rhumatoid polyarthritis of the extracts was compared to that obtained by the same doses of initial polyvalent IgG's. The cumulated results of the two independent experiments (figure 6), show that the effective dose on the development of rhumatoid polyarthritis of the extracts derived from the elution of the NHS-Affiprep gel coupled to DNP-Lysine is ten times lower than the effective dose of Tegeline.

[0068] **Example 7B:** Effect of anti-Tegeline extract and anti-DNP extract on the development of diabetes induced by cyclophosphamide in the male NOD mouse.

[0069] The newly born male NOD mice are injected three times a week during 4 weeks either with Tegeline at a dose of 1 mg/young mouse, or with an anti-Tegeline extract or an anti-DNP extract at a dose of 0.1 mg/young mouse. Development of diabetes is triggered at the age of 8 weeks with two injections of cyclophosphamide (200 mg/kg) within the space of two weeks. Figure 7 shows that the percentage of diabetic mice [rate of sugar in blood higher than 3g/l) is significantly decreased in the group of NOD mice injected with Tegeline (14%) and in the group injected with the anti-DNP extract (21%) but not in the group injected with the anti-Tegeline extract as compared to the non treated group (68%)].

[0070] These indications are not exclusive and could be extended. Said extracts are compounded with a pharmaceutical carrier that is adapted for intravenous administration, with conditioning either in

lyophilized form, or in liquid form or by any other way of administration (IP, ID, IM) depending on the searched for indications.

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[0071]

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Claims

1. Ig extract characterized in that it reacts with at least one component selected from IgM's, IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 20 with respect to the activity of the initial polyvalent Ig's, and in that it does not react with tetanic toxoid and HBs antigen with a ratio of concentration higher than 5 with respect to the activity of the initial polyvalent Ig's.
2. Ig extract according to claim 1 characterized in that it consists in an IgG or IgM extract.
3. Extract according to one of claims 1 and 2 characterized in that it reacts with a component selected from IgM's, IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 40 with respect to the activity of the initial polyvalent Ig's.
4. Extract according to one of claims 1 to 3 characterized in that it reacts with at least one of the autoantigens selected from myosin, actin, tubulin, and the basic protein of myelin (MSP) with a ratio of concentration higher than 10 with respect to the activity of the initial polyvalent Ig's.
5. Extract according to claim 4 characterized in that it reacts with at least one of the autoantigens selected from myosin, actin, tubulin, and MBP with a ratio of concentration higher than 20 with respect to the activity of the initial polyvalent Ig's.
6. Extract according to one of claims 1 to 5 characterized in that it reacts with myosin, actin, tubulin and MBP.
7. Extract according to one of claims 1 to 6 characterized in that it reacts with a component selected from IgM's, IgG F(ab')2's and DNP haptene with a ratio of concentration higher than 40 with respect to the activity of the initial polyvalent Ig's, with myosin, actin, tubulin, and MBP with an average ratio of concentration higher than 20 with respect to the activity of the initial polyvalent Ig's.
8. Extract according to one of claims 1 to 7 characterized in that it reacts with IgM's and IgG F(ab')2's.
9. Extract according to one of claims 1 to 7 characterized in that it reacts with DNP haptene and in that it does not react with IgM's and IgG F(ab')2's.
10. Process for the preparation of Ig extracts characterized in that it comprises the following steps:
    a) preparation of an insoluble support on which a component selected from IgG's, polyvalent IgM's and DNP-Lysine is grafted,
    b) adsorption of polyvalent Ig's on the support obtained in step a),
    c) elution of the Ig's retained on the part of the immunoglobulins linked to the support so as to collect the extract connected par idiotypical interactions IgG-IgG or IgM-IgG; or elution of the extract that interreacts with DNP,

d) selection of the extracts presenting a reactivity towards IgM's, IgG F(ab')2's or DNP haptene, none or little reactivity towards nonself antigens and/or a polyreactivity towards given autoantigens,

e) selection of the extracts presenting a proliferation lymphocyte inhibitor activity in mixed culture with an efficacy that is 10 to 50 times higher than TEGELINE®.

11. Process according to claim 10 characterized in that the absorbed Ig's consist of IgG's or IgM's.

12. Process according to one of claims 10 and 11 characterized in that the Ig extracts are prepared from polyvalent Ig's or any other intermediate extract obtained during the process of preparing IgIV's for therapeutic use.

13. Process according to claim 12 characterized in that the polyvalent Ig's used for preparing the extracts consist of IgG's or IgM's.

14. Process according to one of claims 10 to 13 characterized in that step d) comprises determining the ratio of concentration of antibodies that react against IgM's, IgG F(ab')2's or DNP hapene that are used for purification.

15. Process according to one of claims 10 to 14 characterized in that step d) comprises a determination of the reactivity for tetanic toxoid and the antigen HBs by taking the concentration ratio as control value.

16. Process according to one of claims 10 to 15 characterized in that step d) comprises an ELISA test carried out on a panel of autoantigens selected for example from actin, myosin, MBP and tubulin.

17. Process according to one of claims 10 to 16 characterized in that step d) comprises a competition test to control the neutralizing activity of the extracts towards antibodies originating from the serum of patients suffering from autoimmune diseases.

18. Process according to one of claims 10 to 17 characterized in that step d) comprises a test of inhibition of the mixed lymphocylar reaction with human cells to control the reactivity of purified Ig's.

19. Process according to one of claims 10 to 18 characterized in that step a) consists in grafting IgG's, polyvalent IgM's or DNP-Lysine on a solid support for example on a Sepharose® , Trisacryl®, Affiprep®, Affigel® gel, on gels activated with CNBr, NHS or $C_5H_8O_2$ (glutaraldehyde).

20. Process according to one of claims 10 to 19 characterized in that the Ig's deposited on the solid support obtained in step a) are adsorbed either in the form of lyophilized polyvalent IgG's and put back in solution, or are in liquid form, or in the form of intermediate extracts obtained in the course of a process of preparing polyvalent IgG's in phosphate buffer 20 mM comprising NaCl whose concentration can vary from 0 M to 3 M.

21. Process according to one of claims 10 to 20 characterized in that elution of the Ig's retained in step b) is carried out with an ion buffer dissociating the Ag-Ag or Ag-DNP linkage selected for example from chaotropes such as glycine-HCl or sodium iodide (NaI) under conditions allowing the pH to vary, preferably between 2.8 to 4.9, and/or the molarity of the buffer to vary.

22. Process according to one of claims 10 to 21 characterized in that the absorption is carried out under conditions of temperature that vary from 4 ° to 40 °C and in PBS.

23. Process according to one of claims 10 to 22 characterized in that extracts according to one of claims 1 to 9 are selected during step d).

24. Process for the industrial manufacture of extracts having a reactivity towards a component selected from IgM's, IgG (F(ab')2's and DMP haptene, none or little reactivity towards nonself antigens and a polyreactivity towards given autoantigens characterised in that steps a), b) and c) of claim 10 are used while respecting or adapting the parameters used during the preparation of the previously selected extracts of interest.

25. Extracts capable of being obtained by means of a process according to one of claims 10 to 24.

26. Use of an Ig extract according to one of claims 1 to 9 and 25 for the preparation of a medicament.

27. Use according to claim 26 for the preparation of a medicament intended for the treatment of autoimmune diseases, GVH, and/or graft reject after transplantation.

28. Use acccording to claim 26 for the preparation of a medicament intended for the treatment of Kawasaki disease, the treatment of Birdshot retinochoroiditis, possibly in association with corticotherapy, the treatment of certain cytopenia, hemophilia with inhibitors (auto-antibodies anti-factor VIII), and/or to prevent and/or overcome the immunitary reject of cell grafts and/or organ graft and the development of GVH after grafting allogenic hematopoietic cells.

29. Use according to claim 26 for the preparation of a medicament intended for the treatment of neurological diseases, such as Guillain-Barré disease in adult, chronical inflammatory demyelinisating polyneuropathies, dermatomyositis, myasthenia and/or multiple sclerosis.

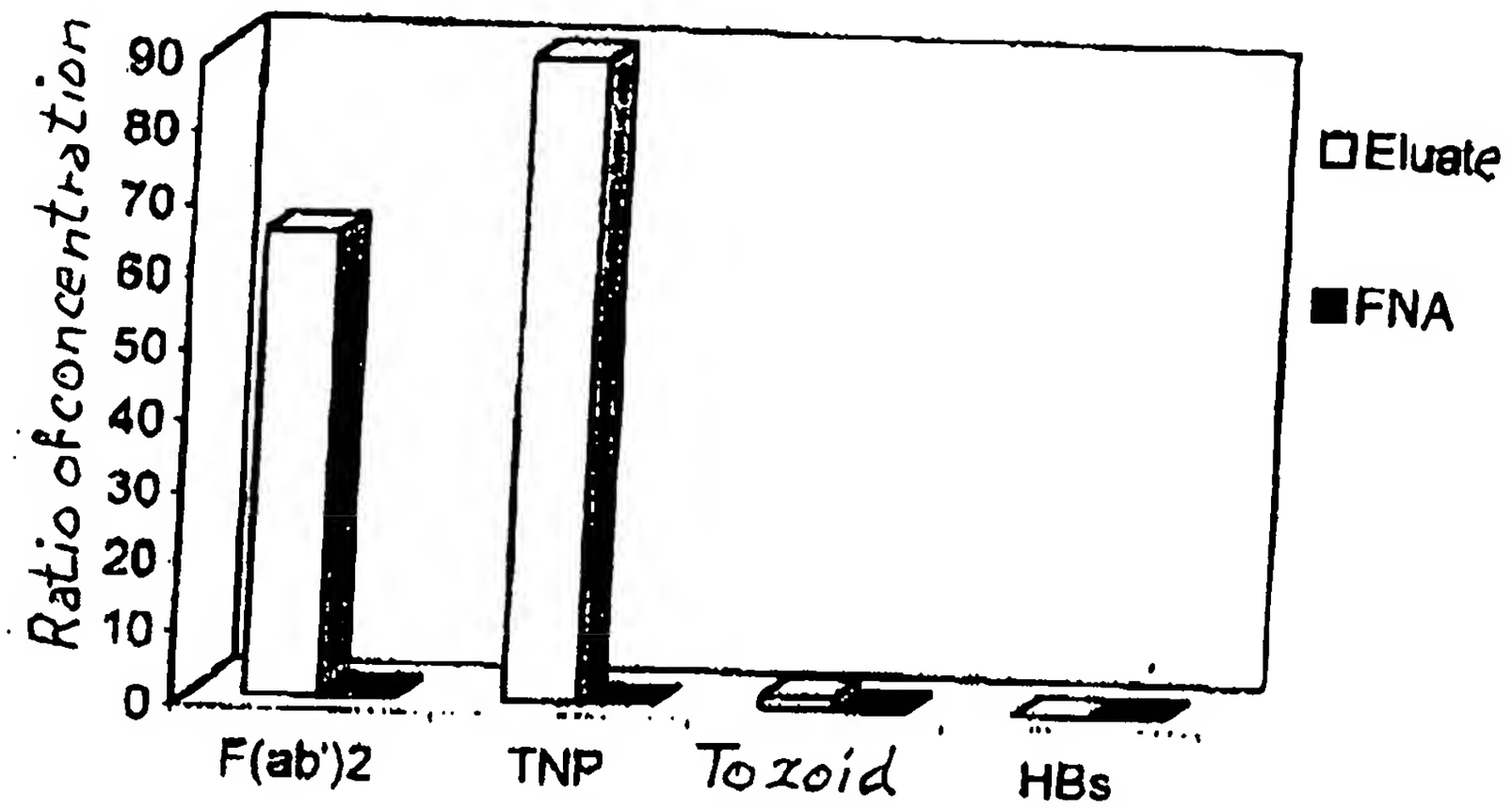


FIGURE 1A

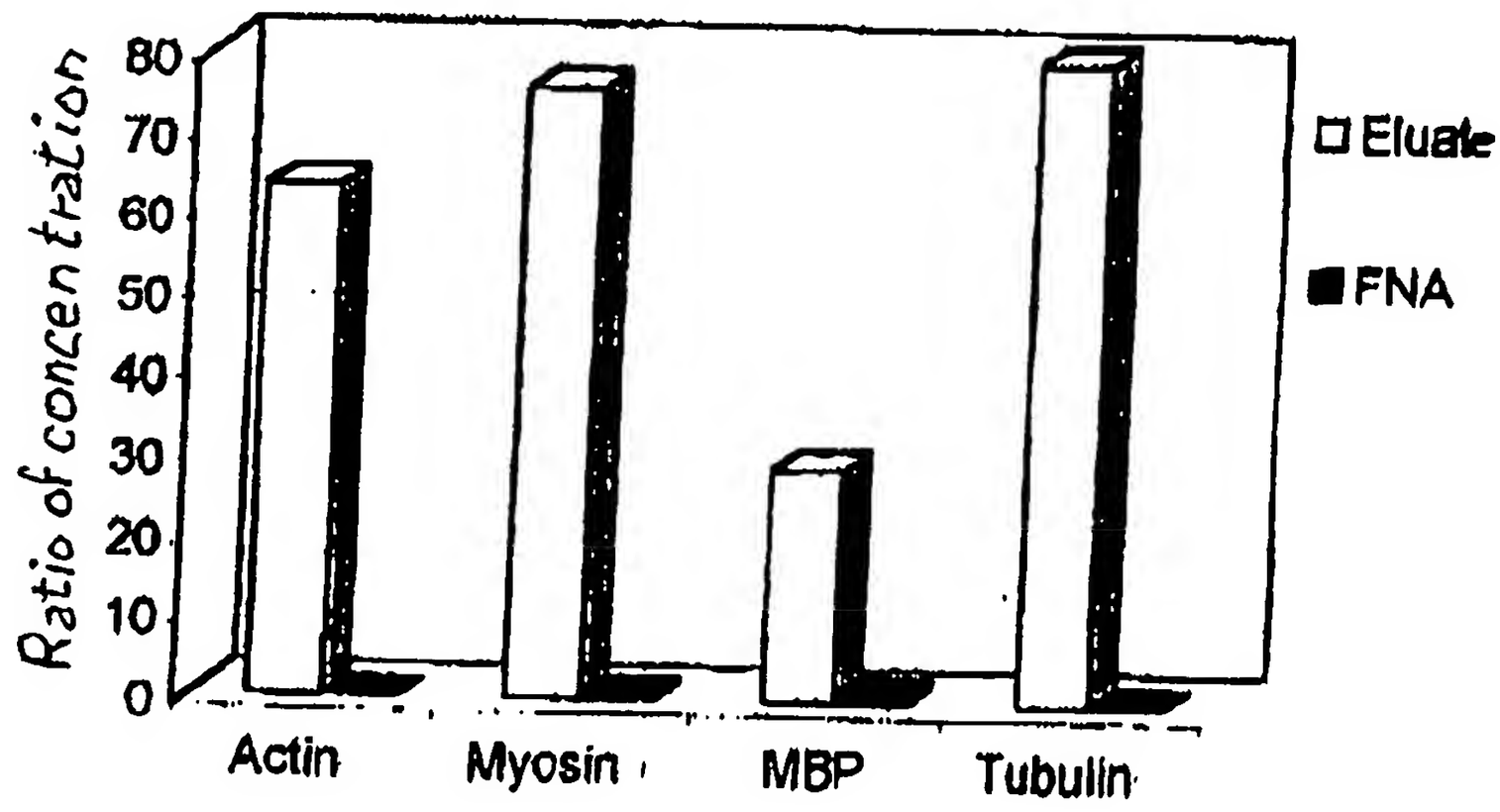


FIGURE 1B

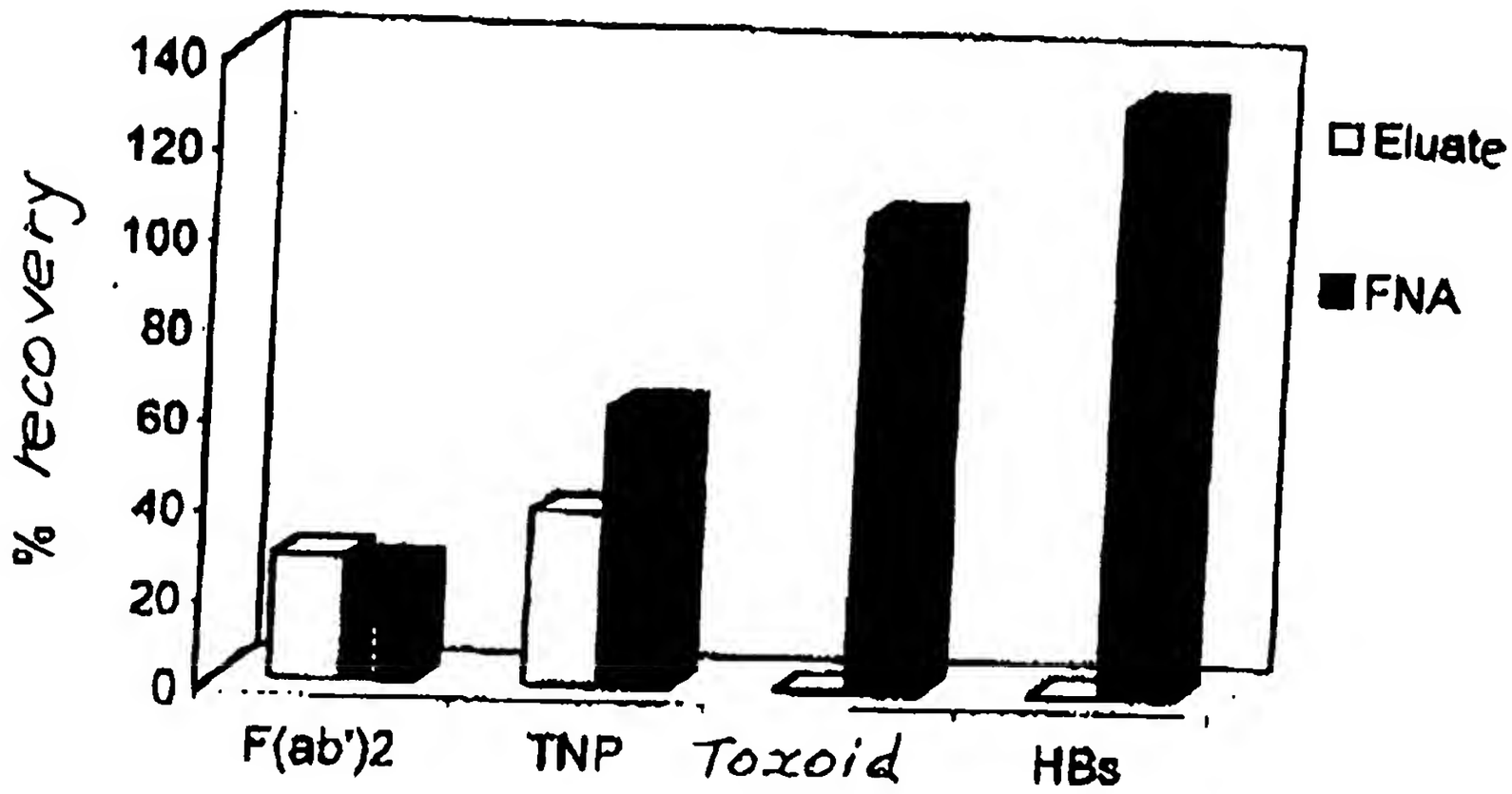


FIGURE 1C

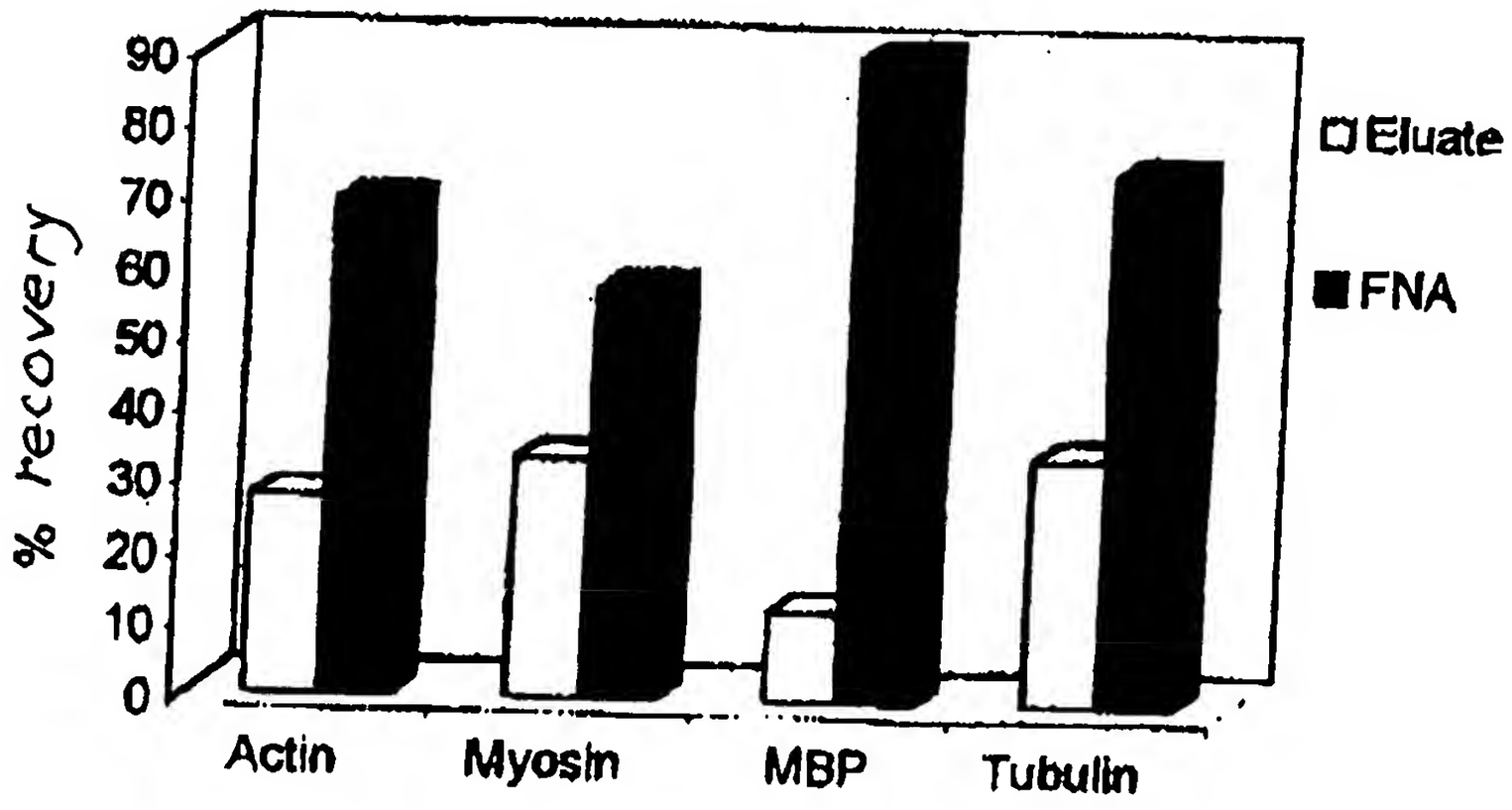


FIGURE 1D

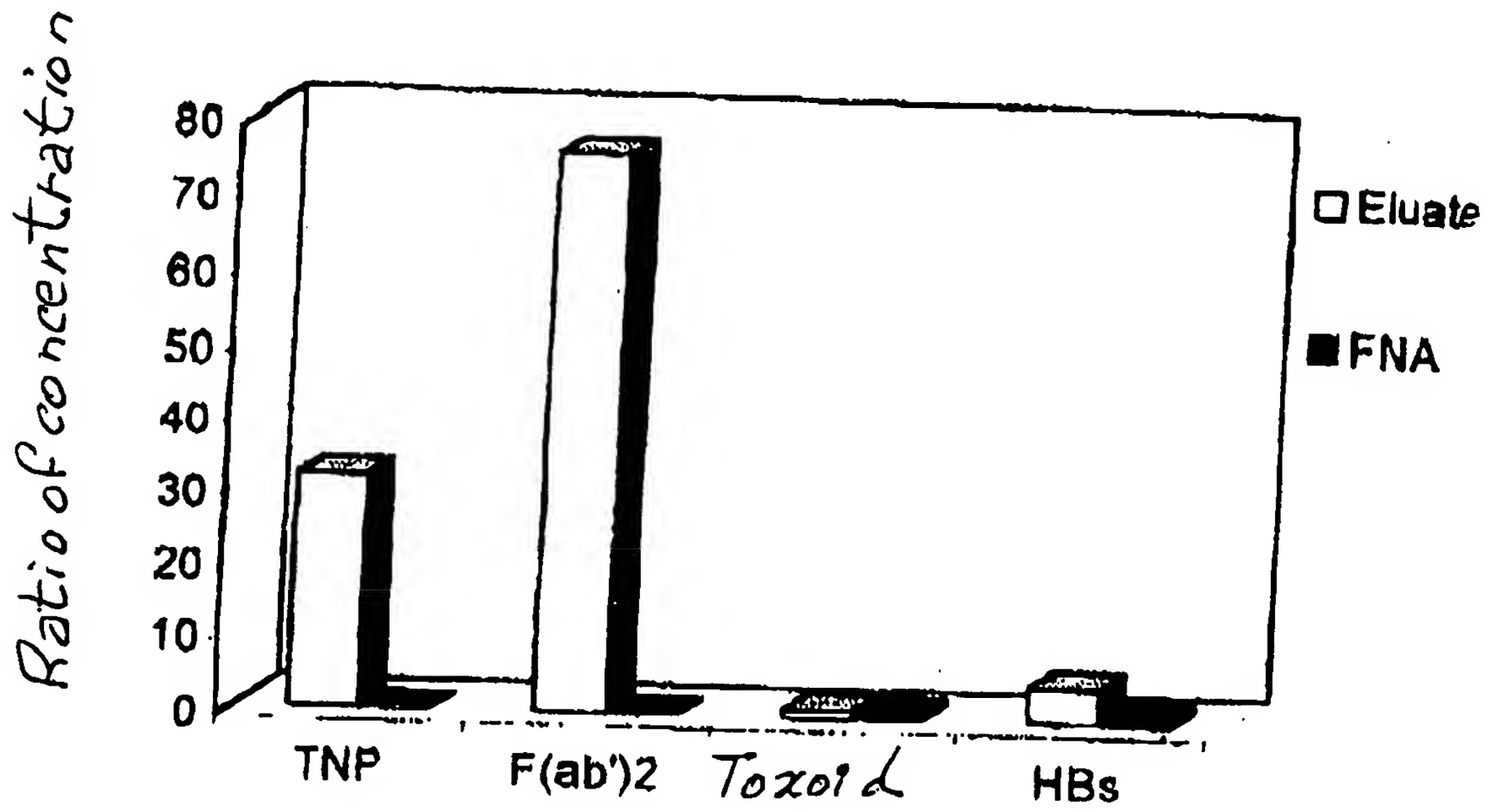


FIGURE 2A

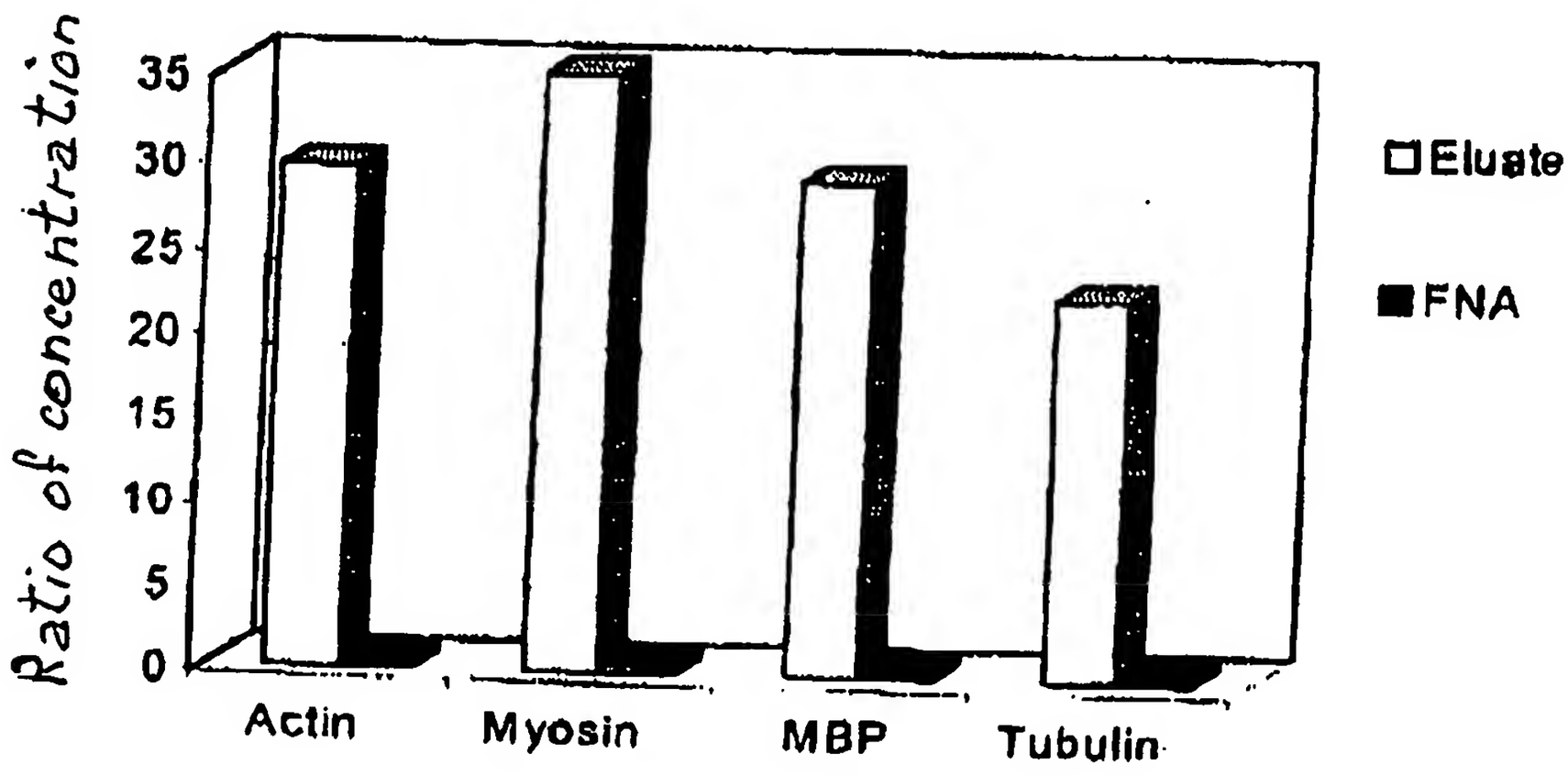


FIGURE 2B

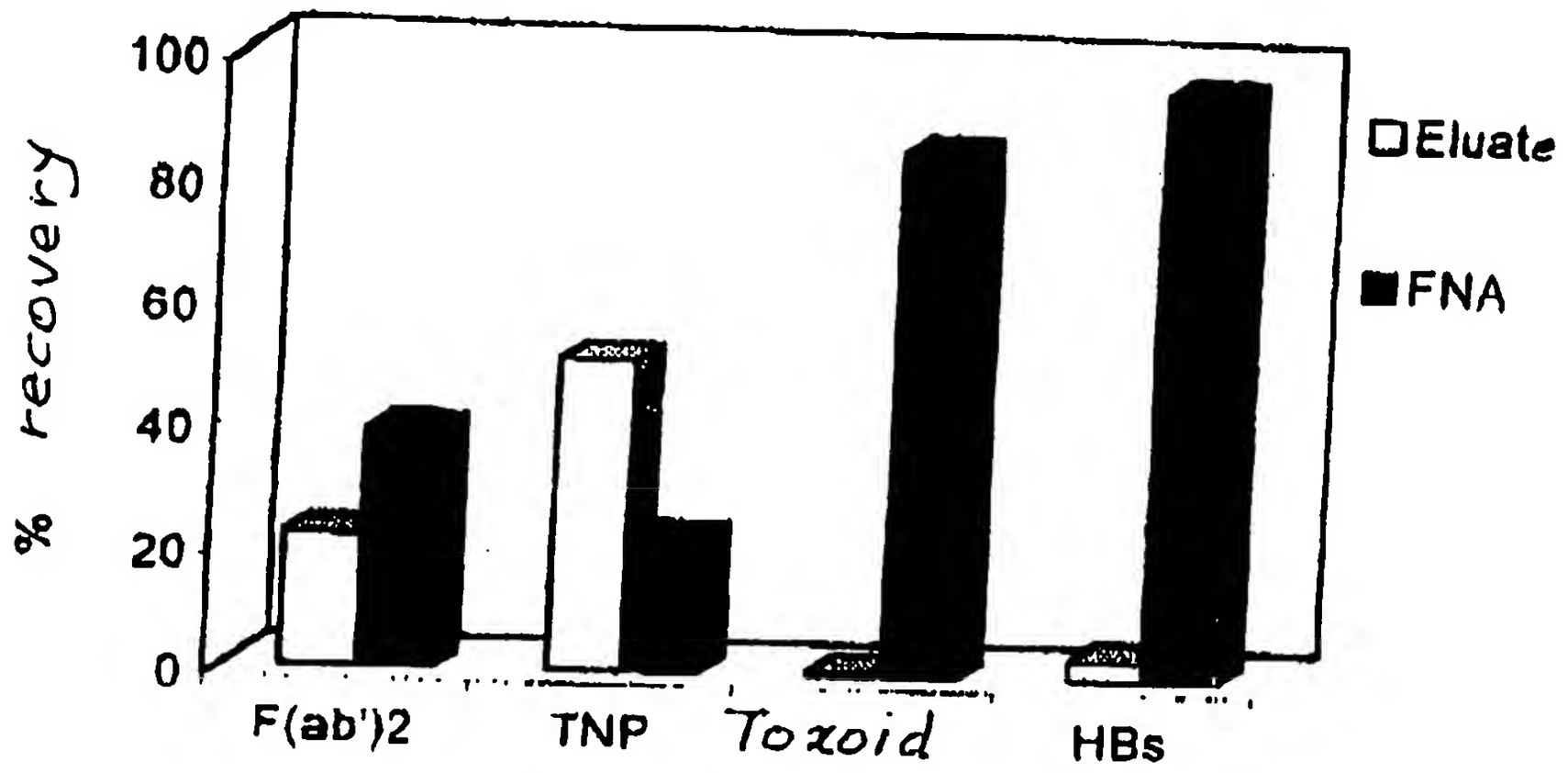


FIGURE 2C

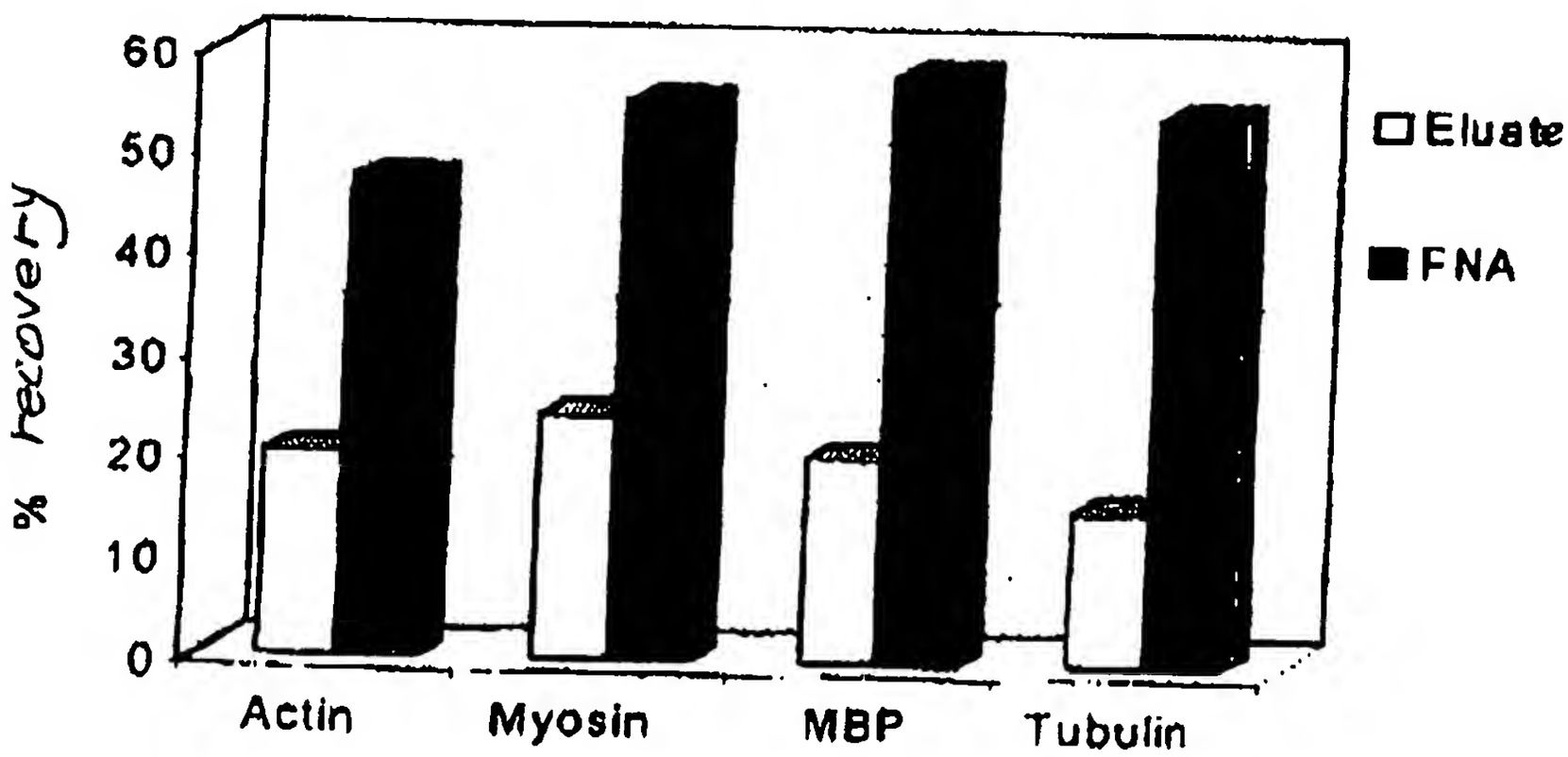


FIGURE 2D

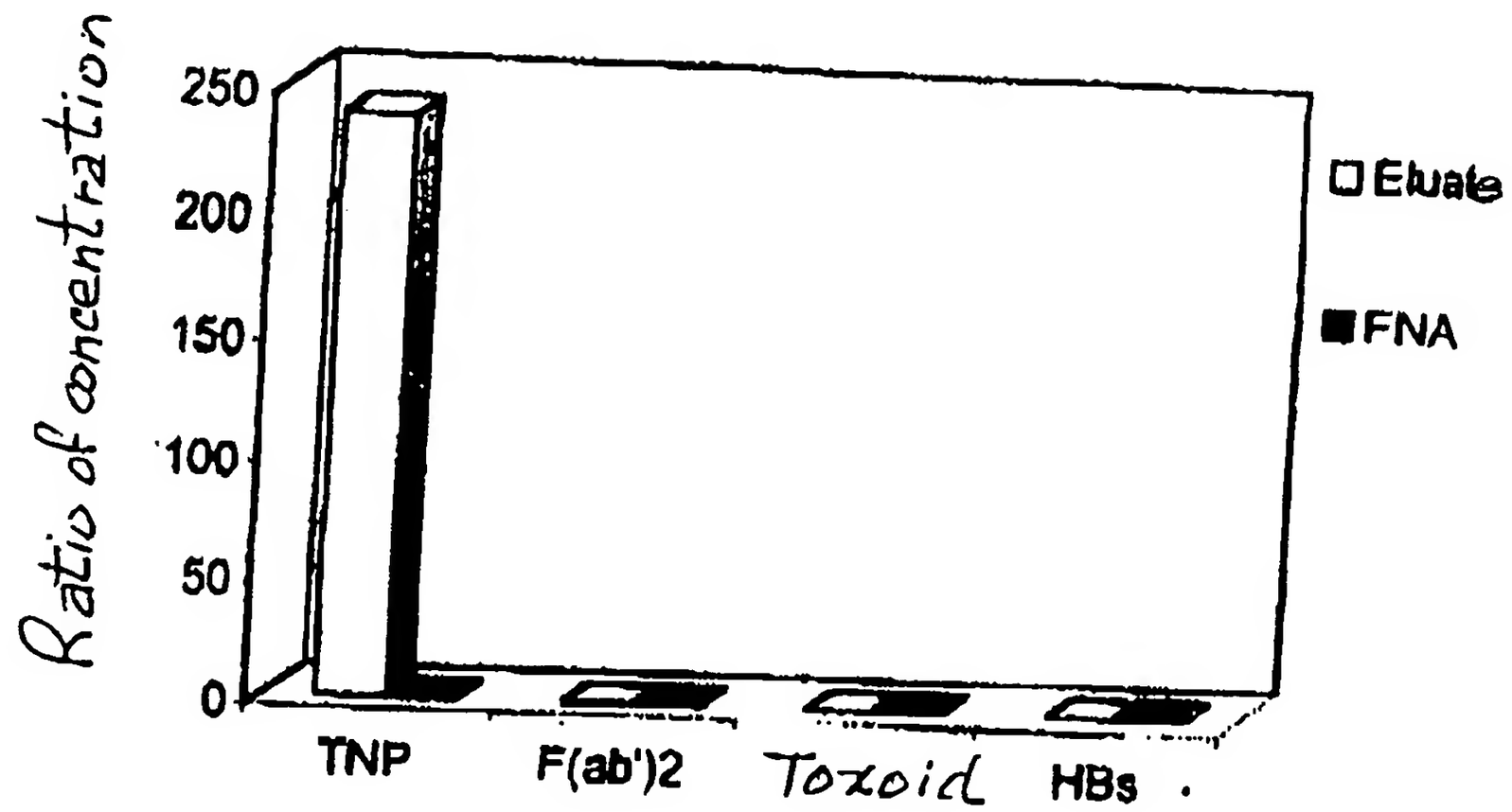


FIGURE 3A

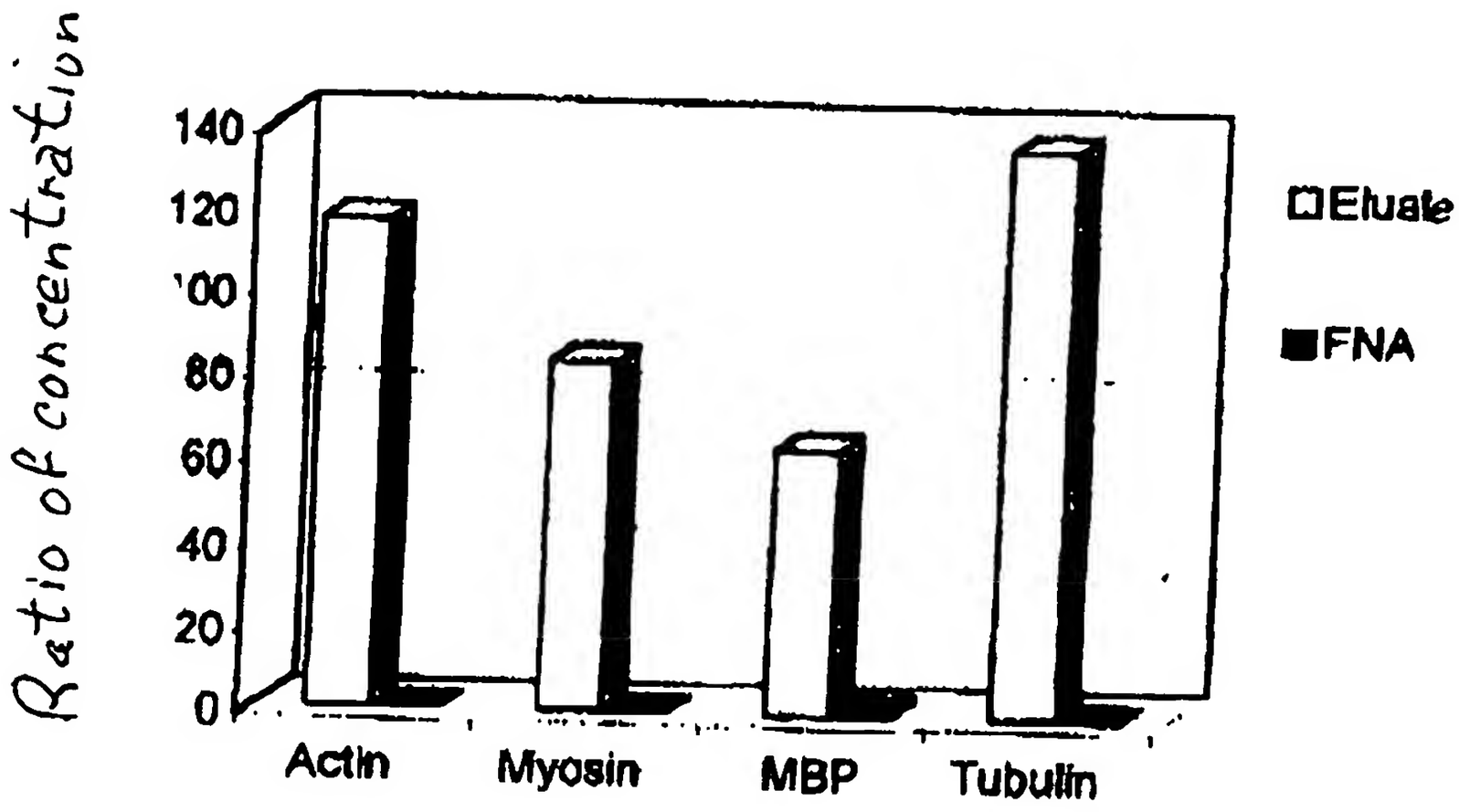


FIGURE 3B

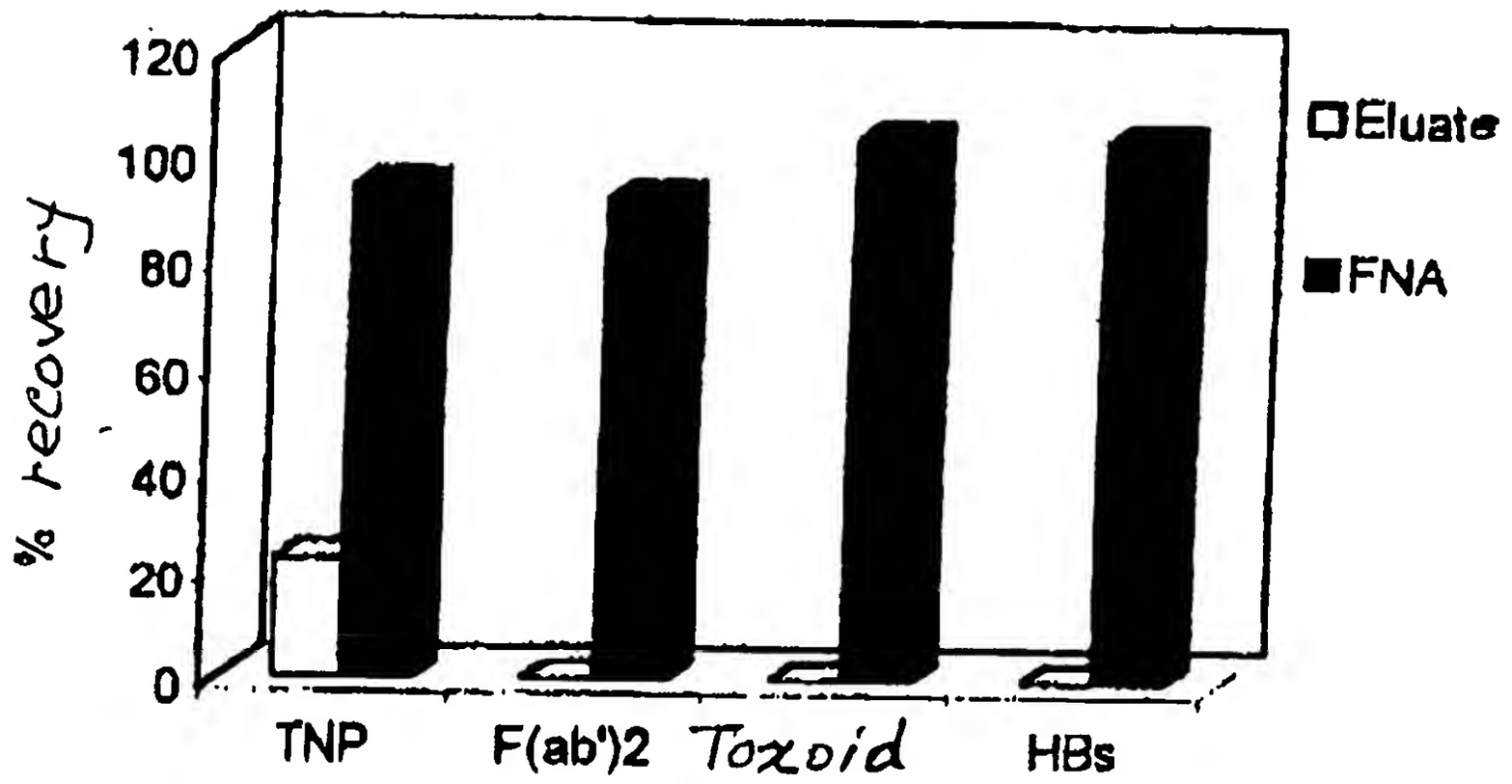


FIGURE 3C

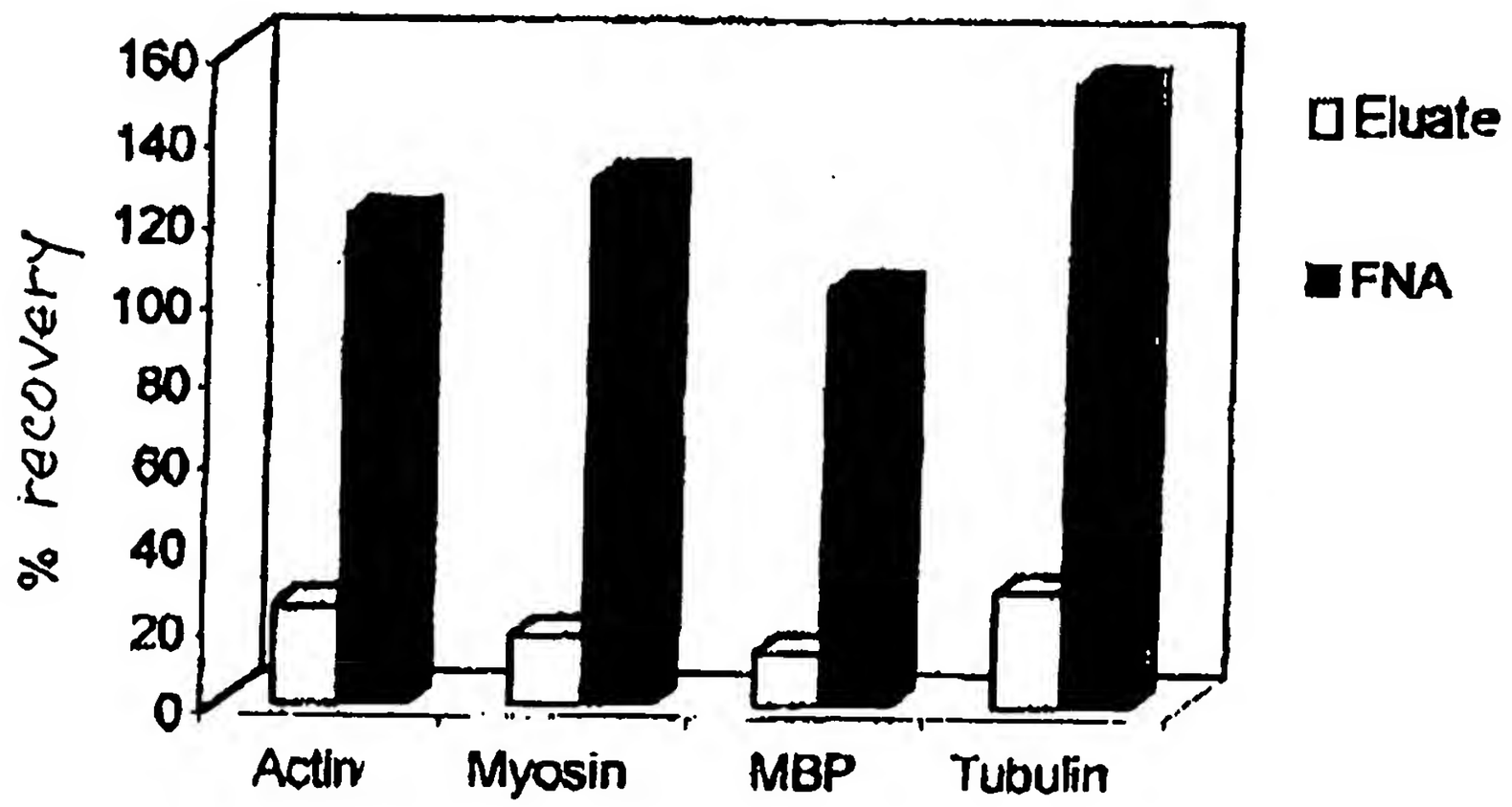


FIGURE 3D

FIGURE 4A

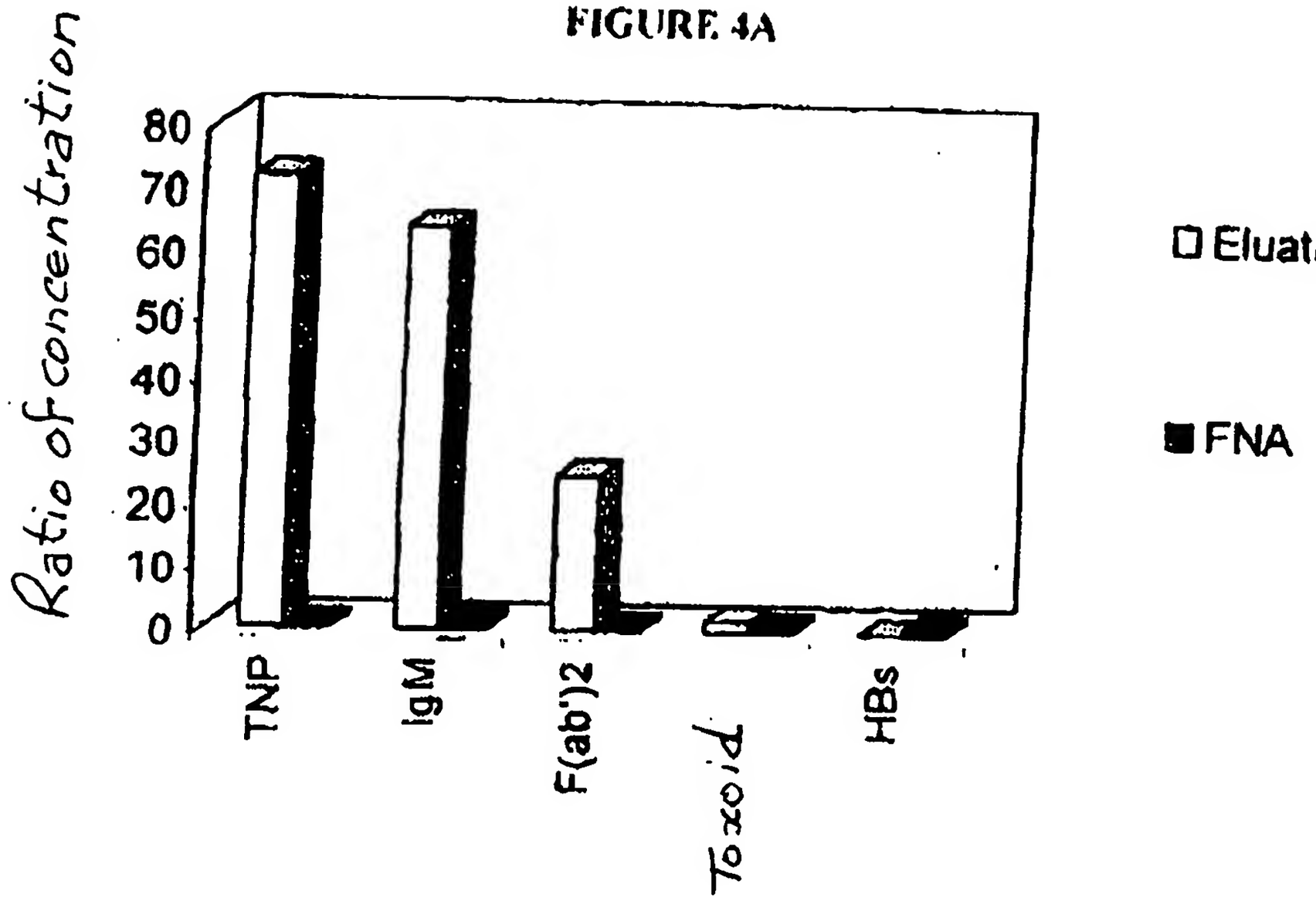


FIGURE 4B

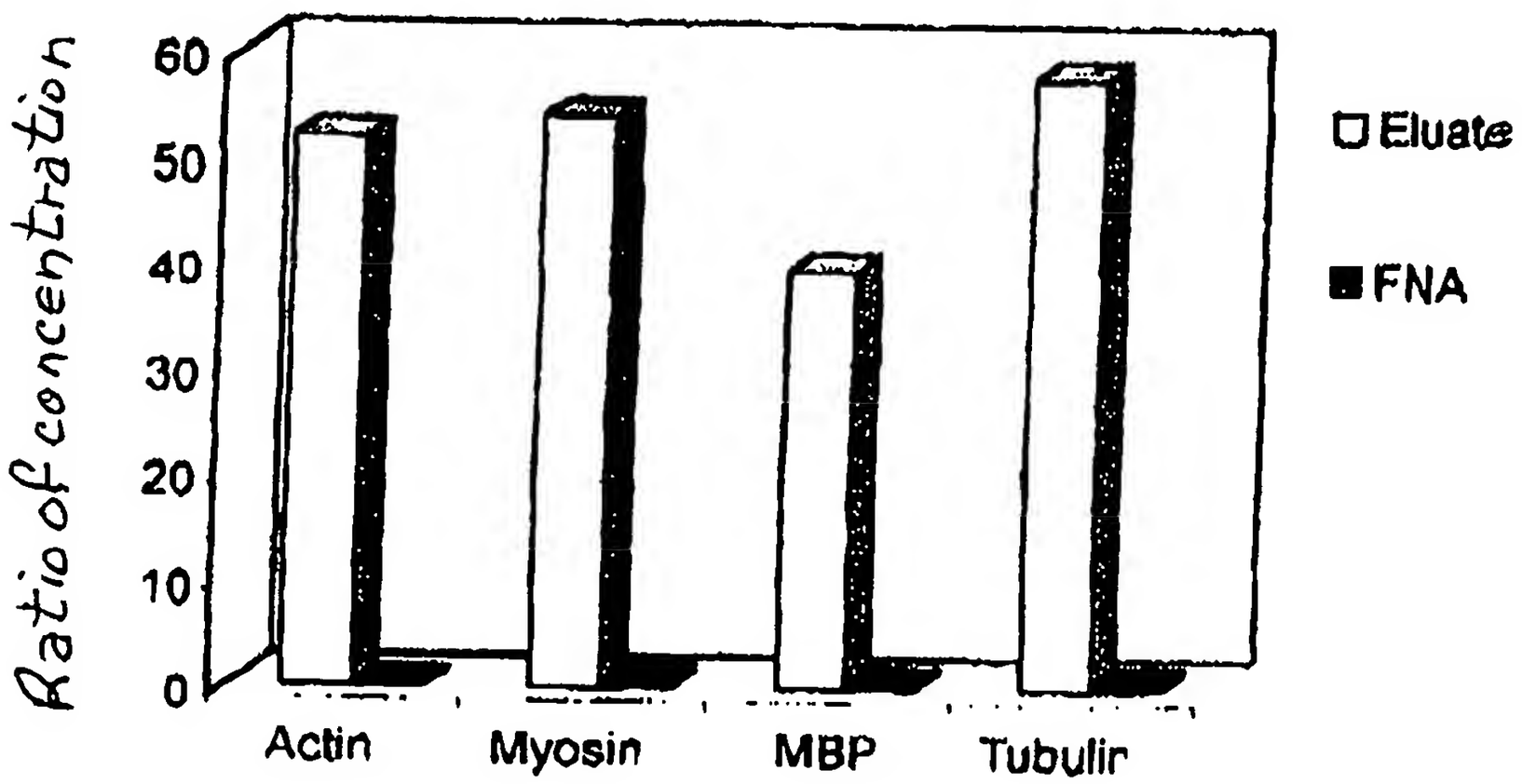


FIGURE 4C

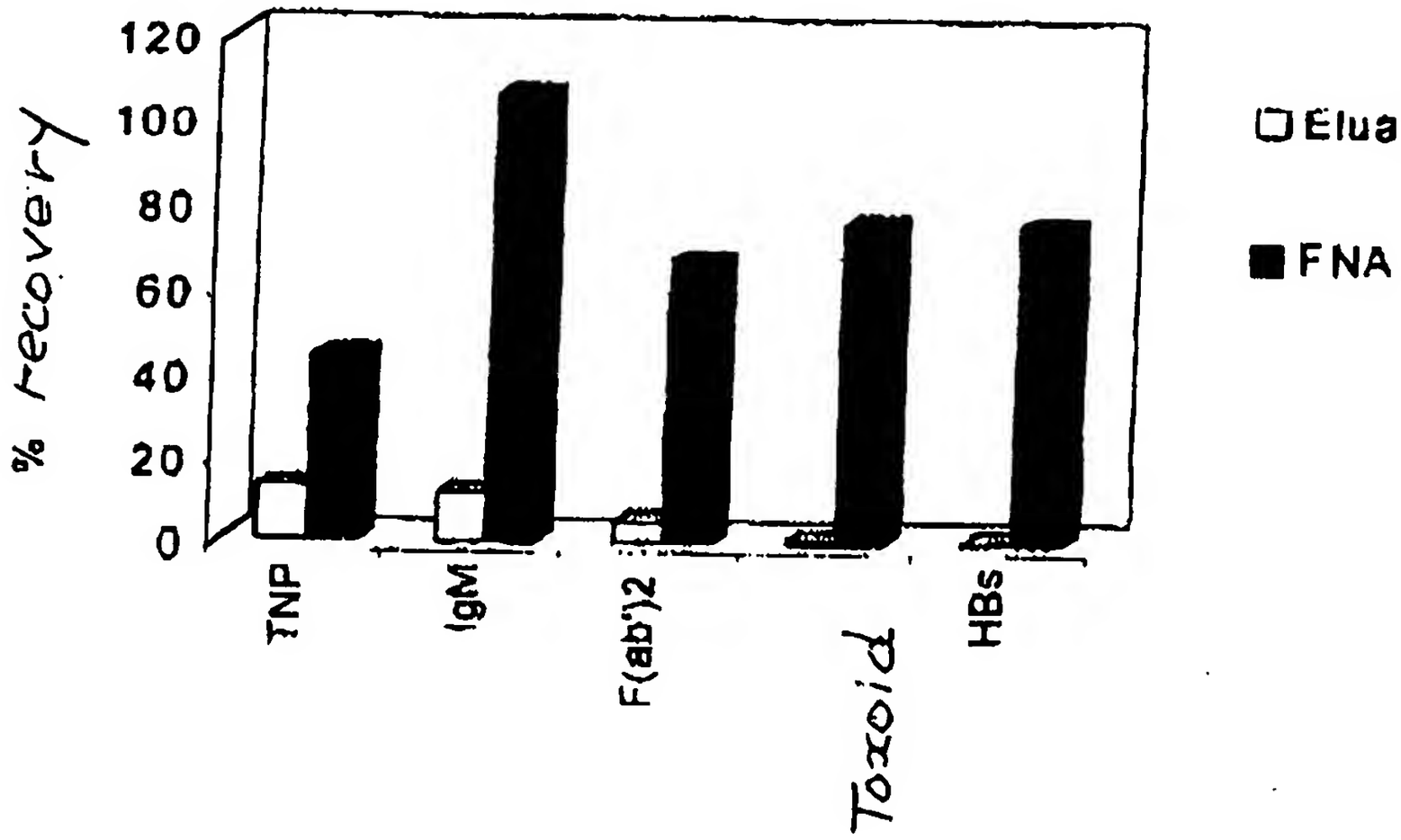
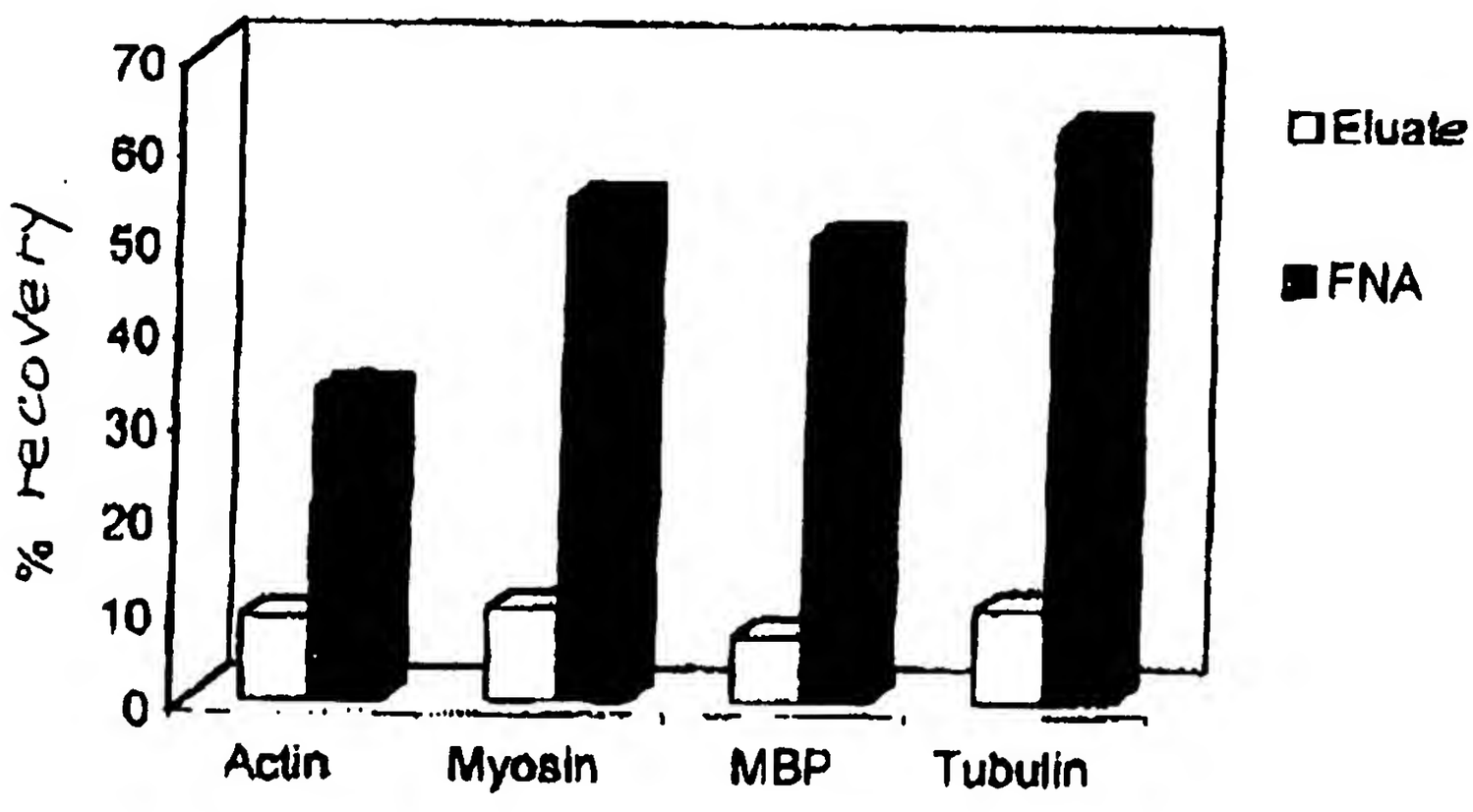
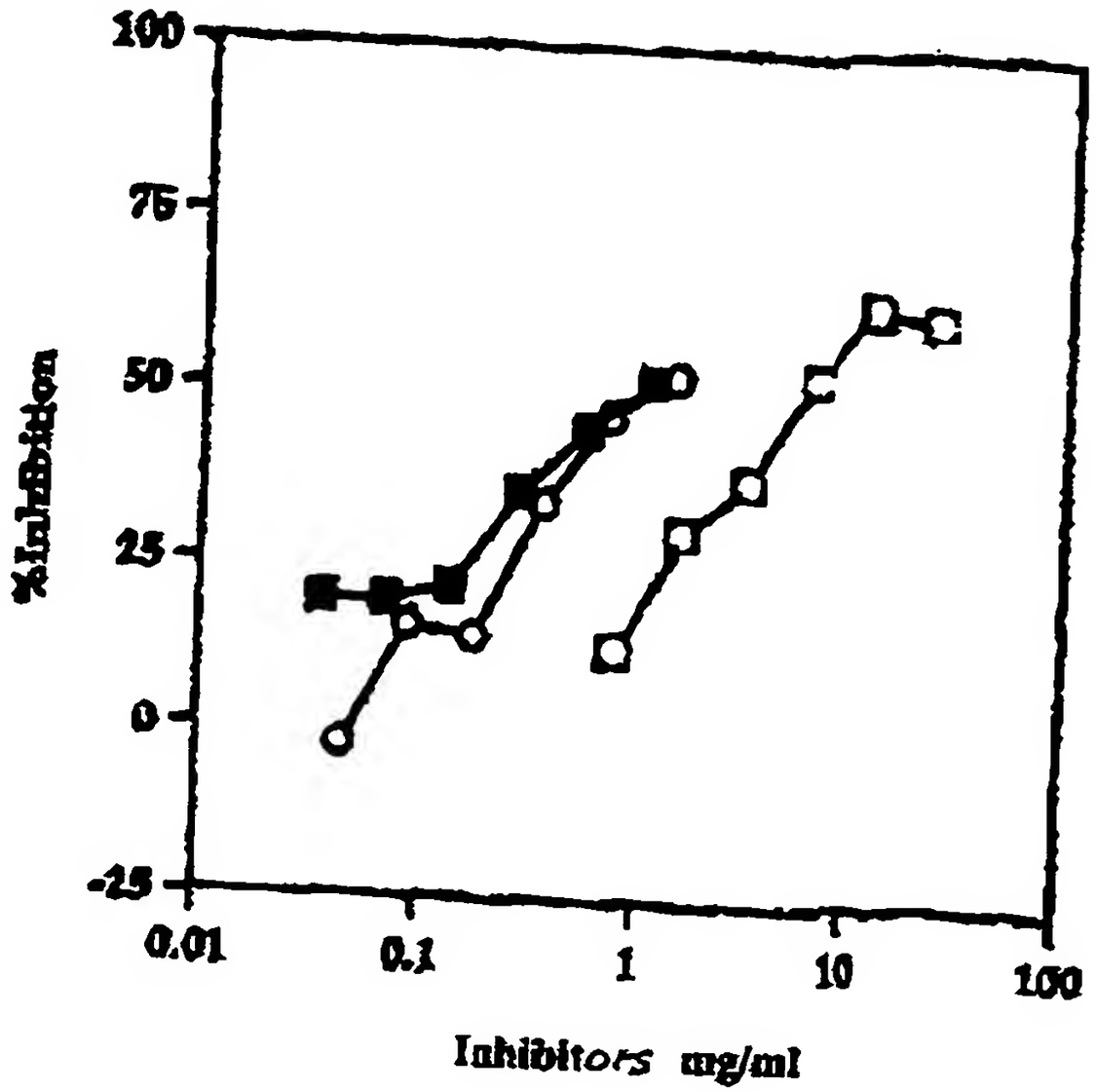


FIGURE 4D





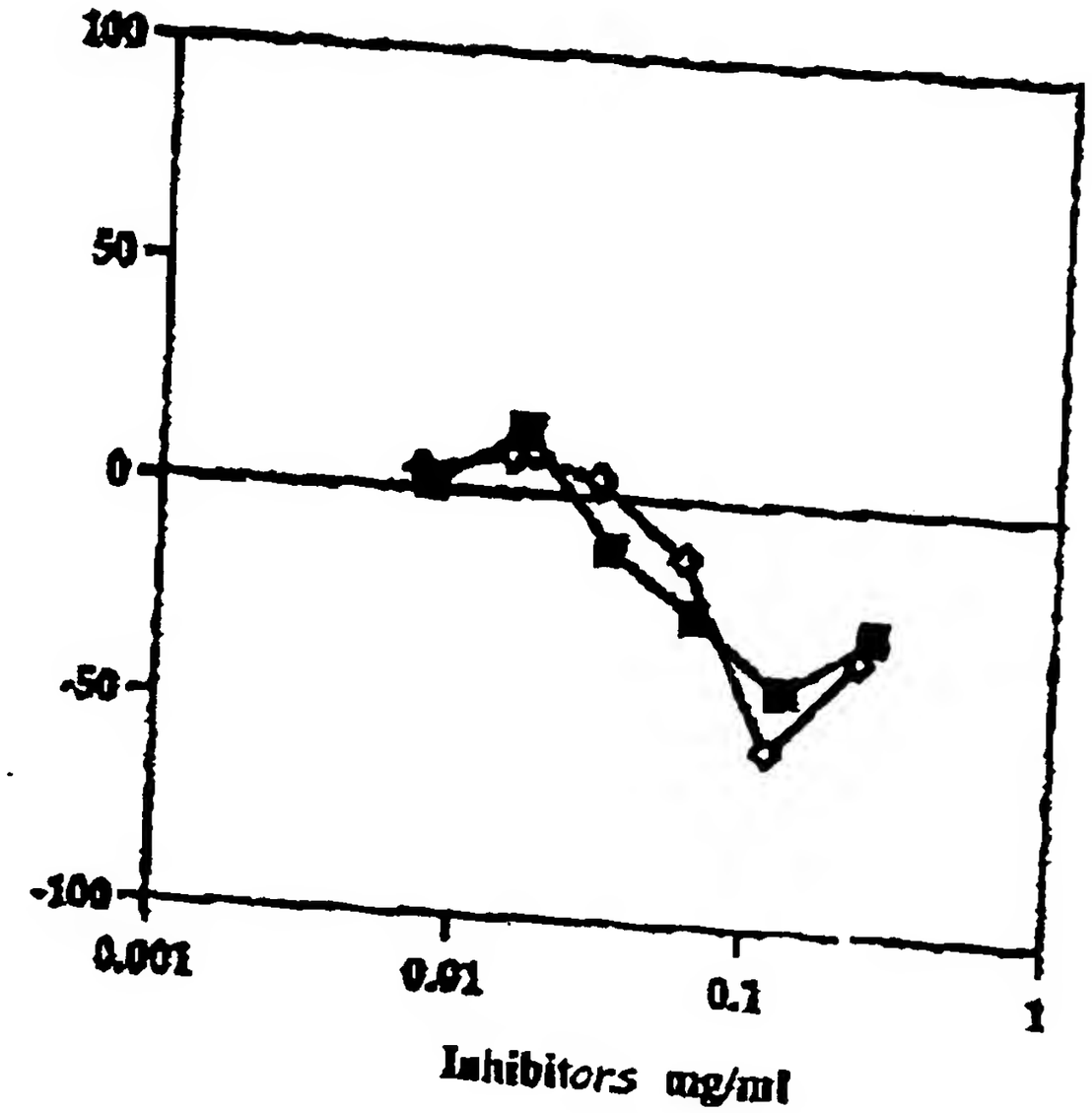


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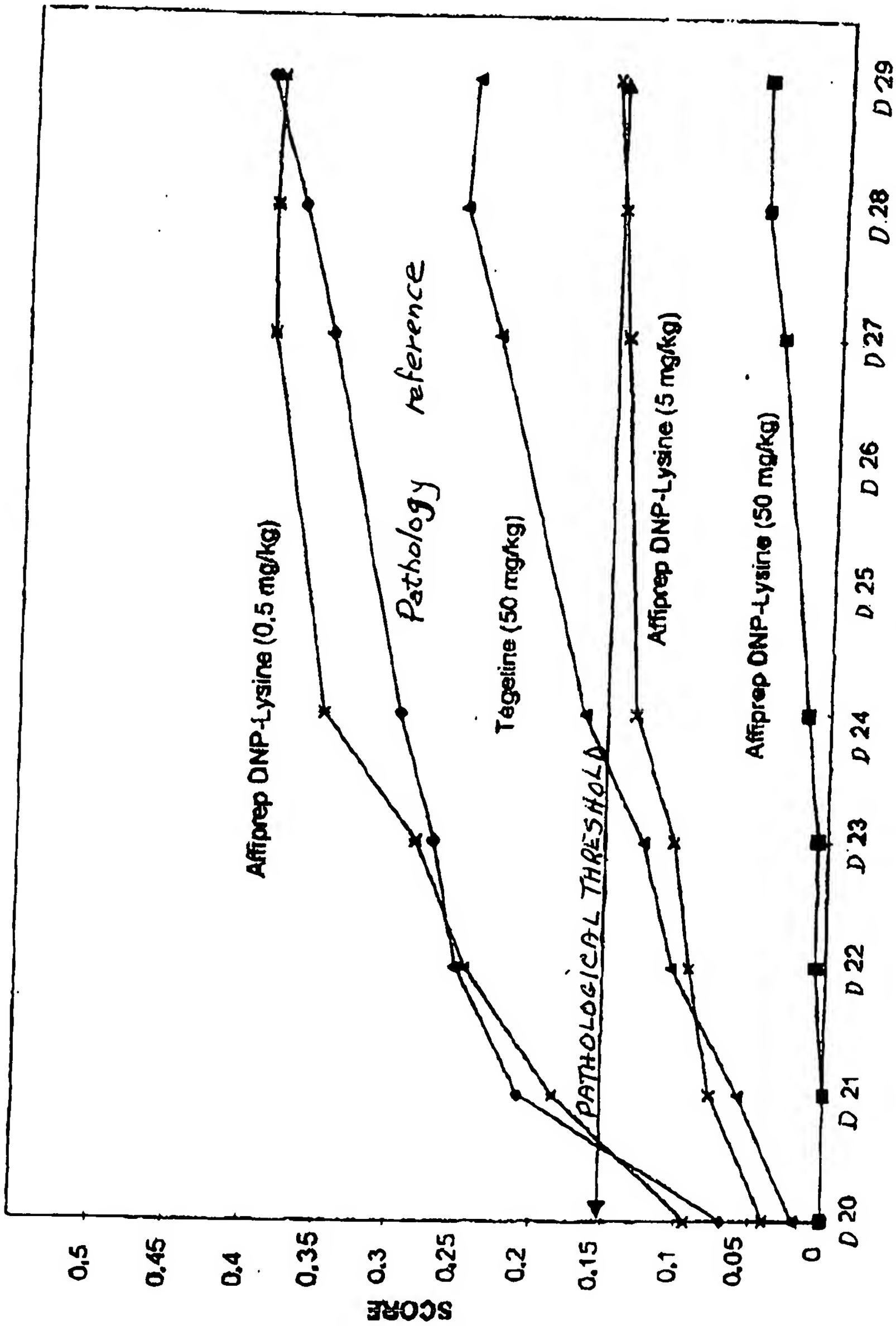
FIGURE 5


Affiprep DNP-Lysine (0,5 mg/kg)
Tegeline (50 mg/kg)
Affiprep DNP-Lysine (5 mg/kg)
Affiprep DNP-Lysine (50 mg/kg)
Pathology reference
PATHOLOGICAL THRESHOLD
SCORE
0
0,05
0,1
0,15
0,2
0,25
0,3
0,35
0,4
0,45
0,5
D 20
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DAY

FIGURE 6

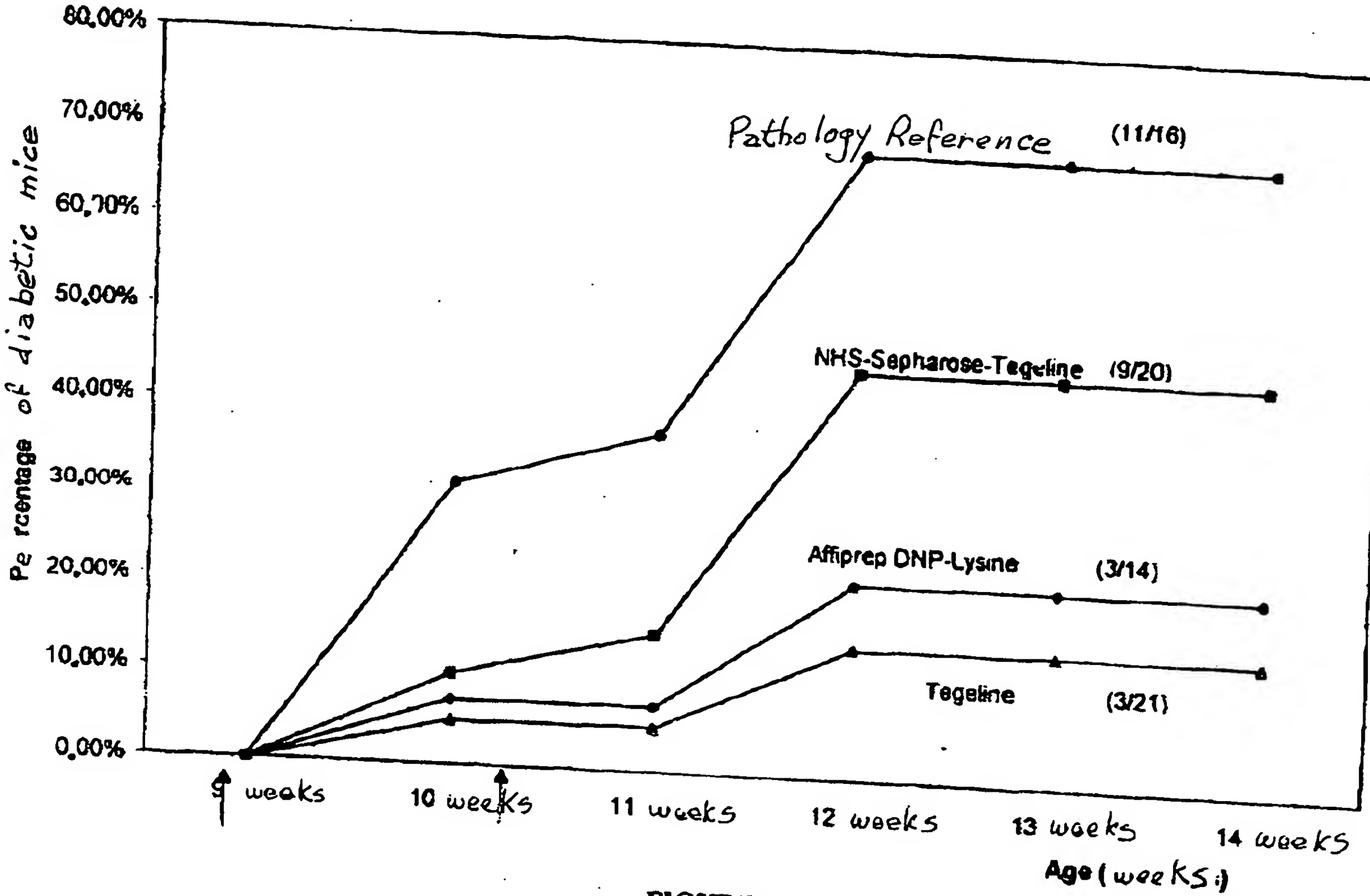

FIGURE 7

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European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 00 40 1601

DOCUMENTS CONSIDERED AS PERTINENT

Category	Citation of the document with indication if needed of the pertinent portions	Claim concerned	Classification of Application (Int. Cl 7)
X	DIETRICH G ET AL: "A V region-connected autoreactive subfraction of normal human serum immonoglobulin G." EUROPEAN JOURNAL OF IMMUNOLOGY, (1992 JUL) 22 (7) 1701-6., xp000877158 *page 1702, left column, line 20 – right column, line 49* *page 1703, right column, line 3 – line 9; figure 8* *table 2* *page 1705, left column, line 43- right column, line 30* ---	1-29	A61J38/385 C076J16/42 C07K16/06 C07K16/18 C07K1/22 A61P37/06
A	JORDAN S C ET AL: "Posttransplant therapy using high-dose human immuno-globulin (intravenous gammaglobulin) to control acute humoral rejection in renal and cardiac allograft recipeidnts and potential mechanisms of action." TRANSPLANTATION, (1988 SEP 27) 66 (6) 88-5 xp000877173 * abstract * ---	26-29	Technical fields searched (Int. Cl 7) A61K C07K A61P
A	PACHECO-GARCIA U ET AL: "Altered	26-29	

	pattern of connectivity in serum immuno- Globulins from pemphigus vulgaris patients." SCANDINAVIAN JOURNAL OF IMMUNO- LOGY, (1999 APR) 49 (4) 424-30., XP000877174 • abstract *	
P,A	FLAN B.: "Fractionation technique and Biochemical properties of IV Ig!. FRACTIONATION TECHNIQUE AND BIOCHEMICAL PROPERTIES OF INTRAVENOUS IMMUNOGLOBULINS (IGIV)." BLOOD THROMBOSIS VESSELS, (OCT.1999) 11/SPEC. ISS. 945-51)., XP000939133 * abstract *	10

The present report was established for all the claims

Search location	Date search terminated	Examiner
THE HAGUE	September 6 2000	Le Flao, K

CATEGORY OF DOCUMENTS CITED

X: particularly pertinent by itself

Y: particularly pertinent in combination with another document of same catogory

A: technological background

O: unwritten disclosure

P: intercalated document

T: theory or principle at the base of the invention

E: prior patent document, but published on or after filing date

D: cited in the Application

L: cited for other reasons

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&: member of the same family, corresponding document

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